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PHYTOPATHOLOGY

VOLUME XIII NUMBER 10

OCTOBER, 1923

THE MELANCONIS DISEASE OF THE BUTTERNUT (JUGLANS CINEREA L.)[1]

ARTHUR HARMOUNT GRAVES

WITH PLATES XIX AND XX AND 5 FIGURES IN THE TEXT

INTRODUCTION

For about twenty years the writer has been particularly interested in the general pathological condition of the butternut, (*Juglans cinerea* L.). During this period several trees in Connecticut of various ages, on which observations were made from year to year, have finally died, and others have been gradually growing weaker. (Pl. XIX, figs. 1, 2). Previous to a note by the writer, in 1919, (8) there was no mention in the literature of any serious trouble; although the leaf spot caused by *Gnomonia leptostyla* (Fr.) Ces. and De Not., (the perfect stage of *Marssonia Juglandis* (Lib.) Sacc.) has been reported to sometimes cause considerable damage (14, 19), and also the basidiomycetous wood-rotting fungi doubtless do not neglect this tree (14).

A thorough investigation of the cause of the trouble was begun in the summer of 1918, during a survey of diseases of trees in Greater New York. In this region, and in the same year in Pennsylvania and Maryland, the butternuts were in such a weakened condition as to compel attention. At this time the acervuli of an imperfect fungus, *Melanconium oblongum*, were found to be of quite general occurrence on the dead branches, and this organism was therefore tentatively assumed to be the cause of the trouble, particularly when greenhouse experiments with healthy twigs inoculated with the fungus in question gave positive results.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF THE DISEASE

In many of the excellent "tree books" now current, one finds statements like the following:—

"Trees of the same age are apt to vary in vigor and size, dead branches are likely to appear early, and sound trees 8 or 10 inches in diameter are seldom seen;" (5) or,

[1] Brooklyn Botanic Garden Contributions, No. 31.

"While the butternut is often a very attractive tree as seen growing spontaneously, it is not to be depended upon for ornamental planting, as it is very apt to develop dead branches which interfere with its symmetry. Good-sized trees, even when growing under favorable conditions, generally show this disfigurement, and larger trees are very likely to die completely before they attain any great size." (7)[1]

Dr. G. P. Clinton tells us that he has noticed the weakened condition of the butternut in Connecticut for a number of years, and calls attention to it in a recent bulletin (4), citing the work of the writer. Dr. L. O. Overholts, who recently collected the causal fungus in the mountains near Pennsylvania State College, writes that it "certainly has the appearance of being parasitic in this instance." Prof. J. Franklin Collins has noticed sickly trees in Rhode Island, and in 1922 sent us specimens of both perfect and imperfect stages of the fungus. Dr. C. H. Kauffman writes that he has made collections in New York and Maryland; and likewise Dr. A. S. Rhoads, in New York.

This testimony, taken in connection with the observations of the writer in Connecticut, New York, Pennsylvania, and Maryland, and with his study of exsiccati (p. 415) of the fungus in question, i. e. *Melanconium oblongum* Berk., and its perfect stage *Melanconis Juglandis* (E. & E.) comb. nov., on butternut, certainly justifies the conclusion that the disease is widespread, and very probably extends over the entire natural range of the butternut.

The disease is important *per se*, because it eventually brings about the death of the tree, although its progress is slow. During its course, more and more twigs and larger branches are killed, and this means, naturally, a progressive loss in the total photosynthetic activity. Typical, rather young trees, about 30 years of age, have been noted in the forest with approximately one third of all their large branches dead. Such a loss cannot fail to have a reactionary, weakening effect on the whole tree. But many trees have also been observed which are almost entirely dead while still young (10–20 years). Older trees with only a few living branches remaining are common. (Pl. XIX, fig. 2). In general, taking into account the shortening of the life of the tree as well as its slower growth due to this disease, we may perhaps estimate the loss of normal healthy growth of butternut timber to be somewhere between 30 and 50 per cent.

[1] The following extract from a letter from Dr. Robert T. Morris, the well-known nut culturist, is of interest: "Over the greater part of Connecticut when I was a boy we had magnificent butternut trees, and on my country place at Stamford the last one of the majestic ones is now dead. Most of the younger generation died before they were thirty years of age, many of them at fifteen or twenty years."

We do not believe however, that there is danger of disappearance of the butternut, due to the activities of the fungus associated with this trouble, as has been claimed. (13) The disease ordinarily progresses so slowly that there is ample opportunity for reproduction of the tree and thus for perpetuation of the species.

But while the present paper is confined mainly to the disease in its relations to the butternut, the trouble is of vastly greater significance in its potentiality with regard to other species of Juglans. The fungus has been collected by the writer on *Juglans nigra*, one of our most valuable species of hard woods, and preliminary work (p. 431) has shown that other species of Juglans are susceptible.

From a larger, practical standpoint, the economic importance of the disease in the butternut is bound up with the economic value of the butternut tree itself.[1]

Besides its timber, which is much esteemed for certain purposes, the tree has several well-known minor uses (13, 17), so that a disease which seriously threatens it should be regarded as of considerable economic importance.[2]

SYMPTOMS OF THE DISEASE

It should be emphasized first of all that the progress of the disease, in typical cases, is very slow. No sudden wilting of the leaves occurs —nor does any well marked lesion or canker, as in the chestnut blight, develop on infected branches.

From observations extending over a period of three years made on 62 trees in various localities in Connecticut and New York, the course of the disease is essentially as follows: small branches, either from the sides of the trunk, or from a larger branch, die slowly in basipetal succession, the disease to all outward appearances proceeding from the distal end of the branch toward the trunk. If this happens during the

[1] A botanist of some standing, in the course of a conversation with the writer about this disease, remarked that our country could well get along without the butternut. With the same line of reasoning one may argue that many of our native timber trees of secondary commercial importance may be dispensed with, and thus eliminate them, one by one. But we are losing all of our chestnut, a tree of the first magnitude; the white pine, the most valuable coniferous species in the Eastern United States, is seriously threatened; and because of the increasing scarcity of better woods many species formerly considered of minor importance, are coming into service, and have a constantly rising value. Certainly this policy of *laissez-faire* as voiced by our colleague, is greatly to be deplored and is diametrically opposed to the principle of conservation.

[2] Butternut is included in the list of 100 important eastern forest trees in "Important Forest Trees of the Eastern United States." U. S. Dept. of Agr. Dept. Cir. 223. 1922.

growing season, the leaves of the branches in question turn yellow gradually and drop off—in such slow succession, however, as to pass unnoticed by the casual observer—or the gradual death of the branch may occur in the leafless season. The bark of the dying branches changes from its natural dark greenish brown tinge to a reddish brown and finally a dull gray. Soon it becomes studded with small, dark, smooth papillae, which at length burst open exposing the black pustules or acervuli of the fungus, (Pl. XX, figs. 3, a, b, c). A year or so later all that remain of the pustules are small rounded or irregular holes in the loose outer bark (Pl. XX, fig. 7).

The disease is characterized by a succession of new, rather short branches which die in their turn, developing farther and farther down the main branches or leaders. Occasionally a crown of short lived suckers which soon become infected, appears on the trunk itself. In the last stages of the disease the tree is marked by the bare, gray, weathered wood of dead branches in the upper part, giving a characteristic stag-headed aspect (Pl. XIX, fig. 2). At this period, too, basal shoots often arise which soon become infected.

THE FUNGUS

Synonymy

In its imperfect form, the fungus associated with the disease has been known since Berkeley first described it from specimens from the United States in 1874 (2) as *Melanconium oblongum*. This is the stage which is usually found on diseased butternut and other species of Juglans. In 1893 Dr. Shear collected another form in close proximity to the conidial form on dead branches of butternut, and according to a note by him in the herbarium of the New York Botanical Garden, he believed it might be the perfect stage of *Melanconium oblongum*. In the same year Ellis and Everhart named this form *Diaporthe Juglandis* from Shear's material, citing his collection. Since the writer, by culture work (p. 420), has established beyond question that *Diaporthe Juglandis* E. & E. is the ascogenous stage of *Melanconium oblongum*, it seems advisable to assign the fungus to the genus Melanconis of the family Melanconidaceae, for this group is characterized by superficial conidia (except in Valsaria) of the type of Melanconium. *Melanconis carthusiana* Tul. was long ago (12) connected with *Melanconium juglandinum* Kze., which is of common occurrence on *Juglans regia* in Europe. This conidial form is quite similar to *Melanconium oblongum*, but a careful examination and comparison of exsiccati of the two forms has convinced us that they are

distinct. The following new combination is therefore proposed for this American species of Melanconis, and a description is here added to supplement the characterization of *Diaporthe Juglandis* by Ellis & Everhart. (6)

MELANCONIS JUGLANDIS (E. and E.) comb. nov.

Perithecia seated in scanty stroma ⅓ to ½ mm. below surface of bark, in groups of 1–9, usually not more than 5, sometimes 1, globose, or slightly flattened tangentially, black, 500 μ in diameter, (range 441 × 617 μ) their necks black, about 90 μ in diameter, and ordinarily only slightly extruded; asci cylindrical or slightly club shaped, 108 × 11.7 μ (range 90–110 × 10.5–12 μ), with a short stipe about 6 μ long (range 3–9 μ); ascospores hyaline, colorless to light grayish olive—(15, Pl. XLVI), elliptical, with blunt ends or sometimes pointed, 2-celled and slightly constricted at the septum, averaging 20 × 8.5 μ.

Pycnidia developing in culture, black, spherical or irregular, partly sunk in the medium, 150–400 μ in diameter, with a rounded apical pore, and surrounded by a dense felt of mycelium. Pycnospores oblong, hyaline, one-celled, 1 × 3 μ, swelling markedly on germination.

The conidial form is *Melanconium oblongum* Berk. with usually inequilateral spores, averaging 19 × 9 μ, olive gray (15, Pl. LI) under the microscope, but appearing jet black when massed in the acervulus.

Habitat. North America.[1] A slow parasite in wood and bark of *Juglans cinerea* L. and *J. Sieboldiana* L.; also occurring on *J. nigra* L. and *J. regia* L. and perhaps pathogenic there.

HOSTS AND LOCALITIES OF OCCURRENCE

In the following list, which makes no pretensions as to completeness, all specimens except those starred have been examined by the writer. On *Juglans cinerea*.

CANADA: London, May 13, 1892; no definite locality, February 11, 1893, "*Macoun.*"

MAINE: North Windham, July 26, 1923, *A. H. G.*

RHODE ISLAND: Lincoln, September 30, 1922, (perfect stage) *J. Franklin Collins.*

CONNECTICUT: West Hartford, September 24, 1919, *A. H. G.*; Meriden, September 16, 1922; Newington, September 16, 1922; Berlin, September 16, 1922; Hamden, January, 1919; July 2, 1920; October 21, 1920 (perfect stage); September, 1921; January 3, 1922; September 16, 1922 (perfect

[1] In the list of Danish fungi in Dr. Rostrup's herbarium (11) one specimen of *Melanconium oblongum* is included with the statement that it occurred on *Juglans mandshurica* in the garden of an Agricultural High School and is new for Europe.

stage), all *A. H. G.*; North Branford, December 19, 1920, *A. H. G.*; Sharon, July 12, 1920, *A. H. G.*; Southport, September 27, 1922, *A. H. G.*; Norwalk, September 27, 1922, *A. H. G.*

NEW YORK: Farmington, July, 1889, *E. Brown*; Canton, 1857, (no collector given); also a second specimen from this same locality without date or collector; Buffalo, no date, *G. W. Clinton*;* Alcove, August 1893, "with Diaporthe juglandis E. & E.," *C. L. Shear*; Potsdam, no date, (N. A. F. 121); Van Cortlandt Park, New York City, August 16, 1918, *A. H. G.*; Cold Spring Harbor, August 14, 1918, *A. H. G.*; Inwood, New York City, January 3, 1919, *A. H. G*; Baldwin, L. I., December 6, 1921, *A. H. G.*

NEW JERSEY: Newfield, June 1892. (*Fungi Col. 153*).

PENNSYLVANIA: Kennett, September, 21, 1918, *A. H. G*; Shingletown Gap, Center County, September 26, 1922, *L. O. Overholts.*

MARYLAND: Glen Echo, January 9, 1919, *C. H. Kauffman.**

WEST VIRGINIA: Belleville, December 27, 1921, *Lee Bonar.**

WISCONSIN: St. Croix Falls, November 1, 1897, *C. F. Baker.*

IOWA: No definite locality, May 1882, *E. W. Holway.*

On *Juglans nigra.*

NEW YORK: Valley Stream, L. I., April 1921, *A. H. G.*; Baldwin, L. I., December 16, 1921, *A. H. G.*

On *Juglans Sieboldiana.*

CONNECTICUT: Stamford, July 20, 1919, *A. H. G.*; New Haven, July 31, 1920, *A. H. G.*

NEW YORK: Ithaca, campus of Cornell University, March 28, 1904, *C. H. Kauffman*;* Baldwin, L. I., December 16, 1921, *A. H. G*; Brooklyn, (from inoculated seedlings), June 12, 1922, *A. H. G.*

On *Juglans regia.*

CONNECTICUT: Stamford, July 20, 1919. *A. H. G.*

NEW JERSEY: Newfield, 1892, *Ellis* (N. A. F.).

MORPHOLOGY OF THE FUNGUS

Mycelium

In a culture two days old on nutrient medium, the hyphae are hyaline, colorless and copiously branched, the main trunks being from 6–8 μ in diameter (fig. 1, a, b). The cross walls are distinct, the hyphae being often slightly constricted at these points. These numerous branches, constrictions, and also frequent, short, knubbly branches give the young mycelium a characteristic ragged aspect. Various colors and color changes develop under the influence of different culture media, but in old

cultures the prevailing shades are olives and grays. Under the microscope, old mycelium grown in oat agar has a yellow-green shade. In old cultures the main hyphal trunks are of about the same diameter as in the young cultures, but there may be a host of many fine hairlike branches about 2μ in diameter with cross walls indistinct. Between these two extremes, all gradations exist. In connection with the development of conidia and pycnidia in cultures, a smoke gray, fluffy, aerial mycelium

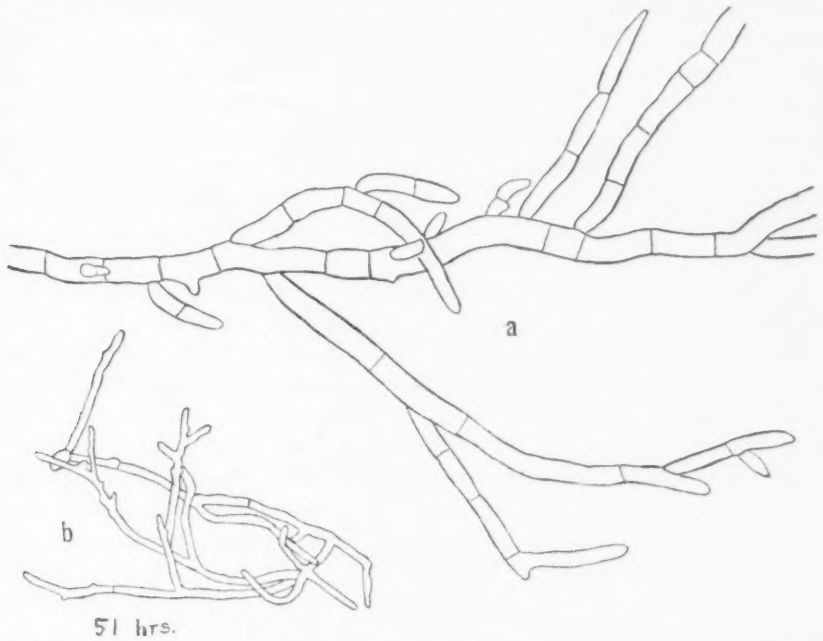

Fig. 1. Mycelium from a young culture: a, $2\frac{1}{2}$ days old, × 410; b, 51 hours old, × 110.

appears, often in cushionlike tufts. This is composed of very fine hyphae essentially similar to the fine branches in the medium. In the bark of the host, the mycelium is even more slender—from 1 to 1.5 μ in diameter.

Conidial Stage

It is this form which is almost invariably seen on dead butternut branches, at first developing small, rounded, pimply, blackened elevations, scattered irregularly about (Pl. XX, fig. 3, a, b) and later bursting through the epidermis and young cork layers with rounded, irregular openings (Pl. XX, fig. 3, c) revealing the pustules of the apparently inky-black conidia. Although pustules appear usually only on the twigs

and small branches, occasionally they are found on the thick bark of the trunk (Pl. XX, fig. 4); and as shown by the figures (Pl. XX, figs. 5, 8, 9) there is much variation in their size. Sometimes they are elongated or run together (Pl. XX, fig. 9). If the weather is damp or rainy, they resemble tiny drops of thick ink, or if the twigs are placed in moist chambers, with the humidity of the correct percentage, the conidia are extruded in tendril form (Pl. XX, fig. 5). We have rarely seen the tendril form in nature, however. With age, the pustules become more flattened like small ink blots on the branch; and at length, after the lapse of a year or even less, are represented merely by rounded or irregular holes in the loose, outer bark, the spores having presumably been washed away or perhaps eaten by insects (Pl. XX, fig. 7). Saccardo (16) gives 20 μ for the length of the conidia. Our measurements average 19.1 × 9.3 μ for 150 spores. The range of all the specimens examined was 14–25 × 4.25–13 μ. This, however, excludes a single giant spore found in the Iowa material, measuring 30 × 13 μ.

In shape, the conidia vary considerably; typically somewhat inequilateral, or with one side curving outward, in some specimens a kidney shape prevails, intermixed with perfectly oval and also symmetrically elliptical forms (Fig. 2, a); ordinarily muticate, sometimes they are bluntly pointed and occasionally terminate at one end in a narrow, short truncate base. Abnormally curved forms also occur, especially in cultures. The optical cross section shows usually a somewhat flattened outline, but it may be quite circular. Crystals of calcium oxalate, such as Johnston (9) records for *M. sacchari*, are common in the pustules.

In color, using daylight illumination, the contents seem to be an olive gray to a deep olive gray (15, Pl. 51) depending upon the amount of light admitted through the diaphragm, but with much darker brownish walls; in fact, it is the wall which gives the dark color to the spore as can be determined by crushing. The contents are then shown to be in reality much paler, though still a shade of gray. Spore specimens which have been kept in glycerine mounts for three years have retained their natural color well.

As to contents, the conidia are densely packed with light-refracting granules, and sometimes contain one droplet or several small ones (Fig. 2, b).

As indicated above, there is some variation in size in the material from different localities, that from Iowa being the largest, averaging 21.0 × 11.0 μ, but taking into consideration all the spore characters, the form seems quite distinct from other Melanconiums.

Pycnidial Stage

This was found only in cultures—in peptone agar in large numbers, and in oat agar (p. 421); in the latter case developing at about the same time as the conidia and surrounded by them. The pycnidia are black to the naked eye, spherical or irregular in shape, 150–400 μ in diameter, with

FIG. 2. Conidia: a, showing different forms, × 600: b, showing granular content or droplets, × 1000.

a rounded apical pore (sometimes 2 pores if of irregular shape), and partly sunk in the medium, being surrounded by a dense felt of pale smoke-gray mycelium. When placed in water, enormous numbers of tiny, oblong, hyaline pycnospores, (Fig. 3, a) about 1 × 3 μ, issue from the openings.

Ascogenous Stage

On Oct. 9, 1920, some of the dead twigs of a living butternut at Hamden, Conn., were observed to be not only plentifully covered with *Melanconium oblongum* pustules, but in and about these same pustules was what appeared to be the perfect stage in cracks in the bark (Pl. XX, fig. 10 and text fig. 4). The twigs were incubated in a moist chamber, and under these conditions, groups of long, black, hairlike structures, the necks of perithecia, grew out from the cracks (Pl. XX, fig. 11). Mature spores, shot out from the asci through these necklike openings,

were caught on slides suspended above, and sowings were made in oat agar on petri dishes under sterile conditions. These cultures matured typical conidia of *Melanconium oblongum* in 22 days. On December 2, the experiment was repeated, and on January 3, 1921, conidia again appeared.

The perithecia found at Hamden, Conn. were compared with the material at the New York Botanical Garden under the name of *Diaporthe Juglandis* E. & E. (p. 414) and found to be identical, with the exception, of course, of the long, extruded necks which had developed under cultural conditions.

On October 7, 1922, we received specimens labelled *Diaporthe Juglandis* E. & E. recently collected on butternut in Rhode Island by Prof. J. Franklin Collins. Examination showed that these also were the perfect stage. In October, 1922, the writer again collected the perfect stage at

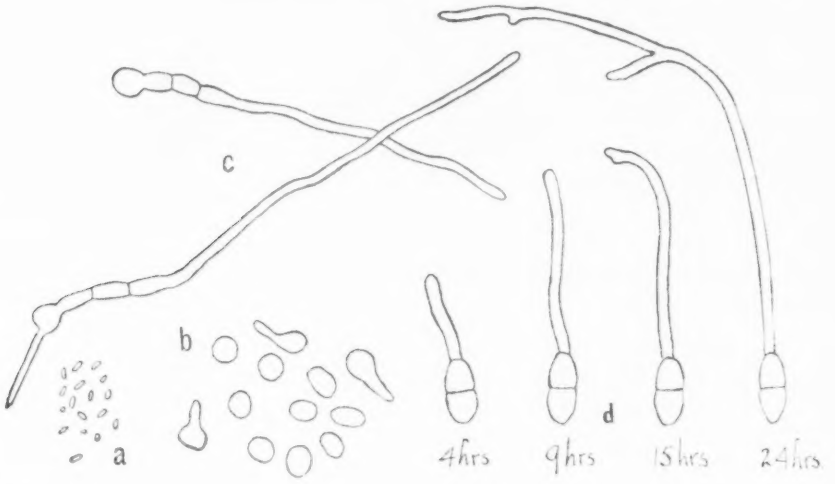

Fig. 3. Pycnospores; a, freshly ejected from pycnidia; b, 15 hrs. later, commencing germination; c, 18 hrs. after a, showing well developed germ tubes, × 500; d, germinating ascospore, × 500.

Hamden, Conn. It is extremely inconspicuous, being located in small cracks in the bark which when not as numerous as in the twig figured (Pl. XX, fig. 10), might pass for old lenticels.

Agar Cultures

Turnip juice agar, oat agar, potato juice agar, peptone agar, and butternut twig agar were all used as media, but for the production of

spores the oat agar gave by far the best results. This was somewhat modified from Dr. Clinton's formula (See Rept. Conn. Agr. Expt. Sta. 1909–10: 760ff) as follows: fifty grams of rolled oats were cooked in 300 cc. of water in a double boiler for about one half hour, then pressed through cheesecloth or a fine wire strainer, 12 g. of agar added, and enough water to bring the whole volume up to 1000 cc. After boiling in the autoclave to melt the agar, titration showed the medium to be nearly neutral. After sterilization, the medium was opaque and nearly smoke gray (15, Pl. XLVI).[1]

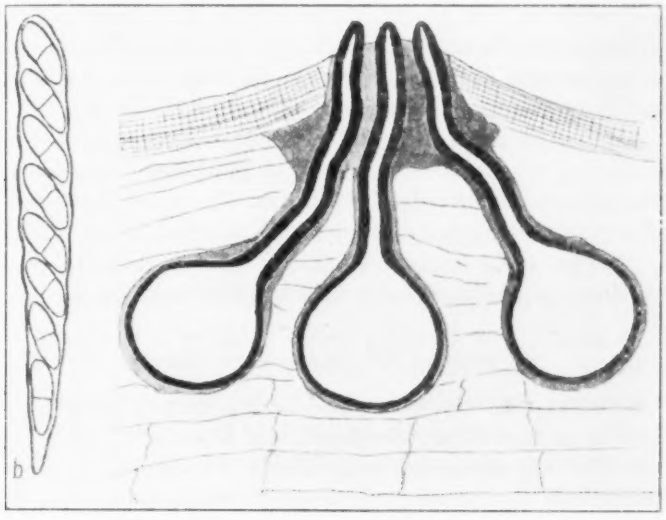

Fig. 4. a, Diagram of perithecia in bark, × 45; b, ascus with spores, × 550.

Cultures were derived from a single spore and obtained by the isolation and transplantation method, as follows. About two days after germination in the isolation petri dishes, the tiny cultures could readily be distinguished by holding the petri dish up to the light, one selected which seemed remote from any impurity, and then its origin from only one spore confirmed by examination of the petri dish directly on the stage of the microscope. Transplantation with a sterile scalpel to a dish of sterile medium was then a simple matter. Contamination in this process was inevitable, but if a considerable number of transplants were made, some would prove pure.

[1]Roman numerals refer to plates in Ridgway's Color Standards and Nomenclature (15).

The fungus shows marked differences of behavior in the different media, but only its characteristics in the oat agar will be described in detail. The young mycelium (3 days old) is an ochraceous-buff (15, Pl. XV), which deepens (8 days) into an ochraceous-orange (15, Pl. XV), and at length (2 weeks) assumes shades varying from olive-ocher to yellowish olive (15, Pl. XXX) mottled with mummy brown (15, Pl. XV). Tisdale (21) notes the presence of green shades in his cultures of Melanconium from tomatoes. Later, the color becomes more uniformly dark, and aerial mycelium, which up to now has been scanty, develops sparsely. In about 3 weeks a curious metallic luster appears on the surface of the medium, the center of the culture becoming nearly black. Small fluffy mounds of a smoke gray (15, Pl. XLVI) mycelium develop, in which the apparently coal black masses of conidia appear, like drops of thick ink. These changes vary somewhat, both qualitatively and quantitatively, and in time intervals, in different cultures, depending perhaps on the thickness of the medium, amount of moisture, temperature, etc.; but the sequence of colors is fairly uniform. In turnip juice agar and in potato agar, the mycelium has a tendency to wrinkle the surface of the medium. It should be noted that in all the cultures the growth was extremely slow, as compared with forms like Penicillium and Rhizopus.

Cultures on Sterile Butternut Twigs

These were cut into sections a few inches long, placed with a little distilled water in test tubes, sterilized, and inoculated with the fungus in the hope that the ascospore stage would develop on them. In some twigs after a little more than three weeks, conidial pustules appeared in the ordinary way, as small black pustules distributed in the bark. In other cases, the bark was fairly well covered with small cushions of mycelium (1–2½ mm. in diameter) of the characteristic olive shades—varying from a light olive gray (15, Pl. LI) to a deep grayish olive (15, Pl. XLVI) when old (Pl. XX, fig. 12). In about six weeks small, inky-black drops of conidia began to appear in these. This difference in behavior of these two sets of twigs was due perhaps to different moisture conditions. No perithecia developed in either case.

Germination of Spores

Conidia germinate readily in oat, potato, or peptone agar in 10–12 hours at laboratory temperature (Fig. 1, b). In tap water the germination is slight—one or two tubes in each drop—and in distilled water only in rare cases can germ tubes be seen.

Pycnospores sown in oat agar commence to swell and eventually assume enormous proportions, compared to their original size. Beginning as very tiny spores 1 × 3 μ, at the time when the germ tube appears they are about 6 μ in diameter, having taken on a more rounded form somewhat resembling yeast plants. Their volume thus increases nearly 40 fold (Fig. 3, a, b). A similar pregermination swelling occurs in the pycnospores of *Endothia parasitica* (1, p. 8), where it amounts to more than 85 times the volume of the resting spore. Germ tubes were put forth from the Melanconis pycnospores in from 15 to 16 hours at laboratory temperature. (Fig. 3, c.)

Ripe ascospores caught by the method described above (p. 421) and sown in oat agar germinated readily in 12–13 hours at laboratory temperature (Fig. 3, d). Germination occurred also in distilled water and in tap water, but it was poor—less than 1 per cent. Mycelium grown in oat agar from these ascospores produced typical conidia of "*Melanconium oblongum*" in from 3 to 4 weeks.

PATHOGENICITY OF THE FUNGUS

Data Based on Field Work

1. The fungus is constantly associated with this disease in the field. Nearly all wild butternuts of twenty years or more are affected to a greater or less extent. Trees of this age which do not show the conidial pustules, or the marks left by them on dead twigs (Pl. XX, fig. 7), are few indeed. The writer has yet to see a tree more than twenty years of age, whose limbs are accessible, on which in some part, the pustules of the fungus or traces of them, do not appear.

2. The proximal limit of the dead area on twigs or branches has been marked in the field and watched from year to year. This method has demonstrated the gradual encroachment of the fungus on healthy tissue, and subsequent appearance thereon of conidial pustules.

3. Records of 31 trees in a forest at West Hartford, Conn., have been kept since 1919, or through four growing seasons. These records show every year an increased number of branches infected with Melanconis. In some cases where the trees are vigorous, the number of healthy branches developing more than offsets those diseased; in others, the reverse is the case, while in others the total leaf area remains about the same from year to year.

4. The case in which the number of branches dying from this disease each year exceeds the number of new shoots developing, is illustrated by a particular tree in Connecticut which has been under observation for the last 16 years, and because it is considered a typical example of the progress of the disease, will be described here in some detail. Plate XIX, figures 1 and 2, which are photographs taken August 6, 1920 and July 30, 1922, show the course of the disease for a two year period. It is evident that the foliage in the later photograph is much more sparse, and a careful examination of the two figures shows that this is due to the death of many of the small leafy branches seen in the earlier photograph. For example, A, a branch of recent origin, formed in a sucker-like way at the base of a limb which has been gradually dying from the tip downward, is entirely dead in 1922. Similar sucker-like branches at B, bearing foliage in 1920, are now dead. At C, where a limb divides into three parts, a conspicuous growth has taken place in the two years, with development of many small branches, but few remain alive, so that the sum total of the foliage in 1922 is less than in 1920. On all dead branches which were accessible the Melanconis pustules were seen. The soil and light conditions for this tree are exceptionally good, so that there is no evident "physiological" reason for its condition. The fungus gained entrance probably through some dead or weakened branch perhaps 20–25 years ago, and the host has been undergoing a slow death ever since. Obviously only a short lease of life remains.

Inoculations of Healthy Twig Cuttings

As reported briefly in the preliminary note on this disease (8) vigorous cuttings of butternut were obtained in March, 1919, from wild trees, brought into the greenhouse and placed in jars of water. Fifty-nine inoculations with Melanconis conidia were made on 19 cuttings. Three more were used for checks, 8 incisions in all being made on these. On May 1 there were 33 infections, 19 of which showed the Melanconis conidial pustules, while 26 of the inoculations had healed and were apparently uninfected. Checks remained uninfected. A similar series on black walnut showed negative results on May 1.

In a second experiment of like nature, using twigs of butternut, Japanese walnut, and black walnut, the results were inconclusive because most of the cuttings soon withered and died. However, the black

walnut unfolded buds and continued healthy for some time, and this experiment is noteworthy mainly for the fact that out of 28 inoculation on this species, 5 developed the conidial pustules of Melanconis.

Inoculations of Forest Trees

1. In the spring of 1919 the branches and trunk of a young tree of butternut, about 5 inches in diameter, breast-high, were inoculated at 10 different points with a pure conidia-bearing culture of Melanconis. No careful record of the results was kept, but the inoculated branches were observed at irregular intervals and were eventually found in many cases to be plentifully besprinkled with the Melanconis conidia. The inoculations in the trunk healed over, and therefore in August, 1922, the tree was cut down to determine whether there was any internal result from these trunk inoculations. The incisions where the spores had been placed were found to be covered with four rings of healthy wood, but it was evident from the appearance of a dark discoloration that the fungus *had advanced both up and down in the trunk along the line of the 1919 ring of wood.* To determine the extent of this advance the trunk was cut into lengthwise and transverse sections which were left lying on the ground. On returning to the spot two months later, large numbers of the conidial pustules of Melanconis were found in the bark of these sections. This behavior of the fungus in the wood is most important to an understanding of its relations with its host, and will be discussed more in detail later.

2. A more careful series of inoculation experiments was commenced in July, 1919, at Hamden, Connecticut. Fifteen trees of *Juglans cinerea* of various ages were inoculated with conidia and the results noted at three inspections in the same year—August, September, and November. Inspections were made also in March, July, and November, 1920; in July 1921; and in July, 1922; so that these experiments cover a period of three years. One hundred inoculations and 20 controls were made.

In table 1 only the inoculations with positive results are given. With 8 exceptions, all others were made on large branches and on trunks ranging from 2 to 11 cm. in diameter, and externally, at least, gave no evidence of infection. During the first month no pustules had appeared, although according to field notes, in No. 62 there was an indication of slight elevations in the bark. By the second month, 3 of the inoculations gave positive results; in the fourth month, 5; at the expiration of one year, 17; two years, 24; and three years, 36. In the spring of 1922, however, a forest fire weakened trees II and III, with the result that the

TABLE 1—*Results of inoculations with Melanconis Juglandis on Juglans cinerea.*

No. of tree	No. of inoculation	Part of tree inoculated	Diameter in cm. at point of inoculation	Time of appearance of spore pustules (shown by + sign)				
				2 mos.	4 mos.	1 yr.	2 yrs.	3 yrs.
II	6	branch	1.4			+	+	+
"	7	"	1.5					+
"	8	"	1.2				+	+
"	9	"	1.3					+
III	10	trunk	2.1					+
"	11	"	2.0					+
"	12	"	2.0					+
"	13	branch	1.0				+	+
"	14	trunk	1.6					+
"	15	branch	0.7					+
"	16	"	0.9					+
VI	39	"	1.8			+	+	+
VII	40	trunk	12.5			+	+	+
"	41	"	12.5			+	+	+
"	42	"	12.0			+	+	+
"	43	"	11.5			+	+	+
"	44	"	11.5			+	+	+
IX	55	branch	0.3				+	+
"	56	"	0.9			+	+	+
"	57	"	0.4			+	+	+
"	58	"	0.4			+	+	+
"	59	"	0.9			+	+	+
"	60	"	0.5			+	+	+
X	61	"	0.8		+	+	+	+
"	62	"	0.4	+	+	+	+	+
"	63	"	0.4	+	+	+	+	+
"	64	"	1.0					+
"	65	"	0.8					+
"	66	"	0.4					+
"	69	"	0.9					+
"	70	"	0.4				+	+
"	71	"	0.5				+	+
"	72	"	1.0	+	+	+	+	+
"	73	"	0.6		+	+	+	+
XI	74	"	0.9				+	+
"	76	"	0.8				+	+
Total infections showing spore pustules				3	5	17	24	36

fungus appeared on nearly every branch, and taking this into consideration, it is fairly accurate to place the amount of apparent infection at about 33⅓ per cent.

Of the 20 controls, four were made on trees II and III on healthy branches which died in 1922 when these trees became entirely infected. This was also the case with one made on tree VII which died completely in 1920, as noted below. The other controls, except in cases where they were too near inoculations, remained uninfected.

Several peculiarities in the results of these inoculations are noteworthy. First of all, in most cases the living bark around the lesion healed promptly, giving the impression that the fungus was not a pathogen at all. By degrees, however, an inoculated twig would turn a reddish brown, and, if in the growing season, would drop its leaves gradually, the pustules finally appearing as small elevations and later breaking through the bark. In such cases, even the callus which had healed over the incisions became covered with acervuli (Pl. XX, fig. 6).

Secondly, in nearly every case except, for example, in tree VII, the spore pustules appeared on *small branches* not more than 2 cm. in diameter—in the majority of cases, 1 cm. or less.

Finally, if the host had already been weakened, the fungus grew rapidly, as in tree VII. Here the organism grew rapidly from the start in the thick, living bark of the trunk, behaving as does *Endothia parasitica* on chestnut, although no spore pustules could be distinguished for about a year. Tree VII was of fair dimensions, about 5 inches in diameter, breast-high, but growing in a dense forest where it had been suppressed and badly diseased at the top, so that it was in a weakened condition. By the end of four months one inoculation, for example, showed 30 cm. of fungous growth upward, 15 cm. downward, and 7 and 20 cm. from the sides of the point of inoculation. In the bark thus killed, spore pustules developed, (Pl. XX, fig. 4), the only case seen where pustules occurred in the thick rough bark of the trunk. The tree died entirely in 1920 directly from the girdling action of the fungus.

In the case of trees II and III, already mentioned as weakened by a forest fire in the spring of 1922, the conidia appeared on all the inoculated branches soon after the fire occurred. A discussion of these peculiarities will be postponed for the present (p. 431).

Inoculations of Seedlings

Although the inoculations on forest trees brought out several new features regarding the operations of the fungus, it was felt that as regards defining its responsibility in the causation of the disease they lacked

conclusiveness. Among other objections which might be brought forward was the fact that in all of the larger trees selected Melanconis already existed on one or more of the branches, although remote from the inoculations. For it had been found practically impossible to locate trees of any size in the forest which did not show the fungus in some portion.

In order to obtain a more clear cut demonstration it was decided therefore to inoculate seedlings that were entirely free from the fungus, and a new set of experiments was commenced on these in December, 1921, in the conservatories of the Brooklyn Botanic Garden. Through the kindness of Mr. Willard Bixby, the well-known Long Island nut-grower and horticulturist, 45 two or three year old Juglans seedlings were secured, distributed about equally among the four species, *J. regia*, *J. nigra*, *J. cinerea*, and *J. Sieboldiana*. These young trees were potted in one-half loam and one-half leaf mold and placed first in a cool greenhouse, and later in one of moderately warm temperature. Under the influence of the latter, by March 1, 1922 all were in full leaf and growing vigorously.

One thing brought out in clear relief by the previous experiments was the indication that *Melanconis Juglandis* is a weak parasite, attacking rapidly only when its host is weakened. It was therefore decided to weaken some of the seedlings of each species, previous to inoculation, in various ways. Thus, two or three of each species, with their roots carefully washed and uninjured, were transferred to pots of clean washed sand; these and all others, with the exception of a first group (Table 2, Group 1) which were treated to optimum conditions of soil, water and light, were placed beneath the greenhouse bench where the light was poor, and watered sparingly. In addition, those in sand were pruned repeatedly beginning March 1, and continuing up to the time of inoculation (Table 2, Group IV). Another group (Table 2, III) was similarly treated except that they remained in good soil. Group II was similar to III except that there was no pruning. No seedlings of *J. nigra* were placed in Group II. In this way four lots of seedlings within each species (with the exception just noted) were developed under the conditions as stated in the first column to the left in table 2.

With one tree for a control in each of the sixteen groups, inoculations with Melanconis conidia were made on May 17, 1922. By June 12 one tree of *J. Sieboldiana* in class II was dead and well spotted with Melanconis pustules (Fig. 5). Table 2 gives the results to Mar. 1, 1923. Plate XX, figs. 8 and 13, from a seedling of *J. Sieboldiana*, show the progress of the disease from one inoculation down the stem to the junction of the

TABLE 2—*Summarized results of inoculations (May 17, 1922) with Melanconis Juglandis on Juglans seedlings.*

Physical conditions	Species	No. of seedlings	No. of inoc.	No. of seedlings showing development of spore pustules				
				Aug. 5	Sept. 1	Oct. 1	Nov. 15	Mar. 1
I Optimum.	*J. regia*	2	3					
	J. nigra	2	3					
	J. cinerea	3	4					
	J. Sieboldiana	2	2					
II Good soil, poor light, little water.	*J. regia*	2	2					
	J. cinerea	3	3			1	1	1
	J. Sieboldiana	2	2	2	2	2	2	2
III Good soil, poor light, little water, pruned.	*J. regia*	2	2					
	J. nigra	3	4		1	1	1	1
	J. cinerea	1	1					1
	J. Sieboldiana	2	2	1	1	1	1	1
IV Sand, poor light, little water, pruned.	*J. regia*	2	2					
	J. nigra	3	3					
	J. cinerea	3	4					
	J. Sieboldiana	1	2			1	1	1
Total		33	39	3	4	6	6	7

branch. The inoculation on the latter has apparently had no effect, but it is only a question of time when the fungus will descend below the origin of this branch and thus cut it off. None of the controls developed the disease, though one tree of *J. Sieboldiana* in class IV, in which the conditions are severe, has died. Although the results are not striking, they point clearly to the conclusion that the fungus is a weak parasite and that its attacks are most marked in a weakened host.

Leaving out the inoculations on healthy seedlings it will be seen that the amount of infection on the weakened seedlings is somewhat over 25 per cent. Incidentally it may be noted that the experiment brings out a marked susceptibility in *J. Sieboldiana*. Just why those in the sand have resisted the fungus so well is not clear. However, the fungus works slowly, as shown in the previous experiment (p. 426).

DISCUSSION OF THE RELATIONS OF FUNGUS AND HOST

The fact that in most of the inoculations on forest trees the incision apparently healed over, led the writer to believe at first that the fungus was not a pathogen, or at least only a very weak one; for one had to admit that it "took" very readily in the living bark of tree VII (p. 427). The comparatively sudden death of whole twigs after two months, followed by an increasing number of dying inoculated twigs and small branches each year up to the termination of the experiments, led very early in the work to a field investigation of the inoculations. It was

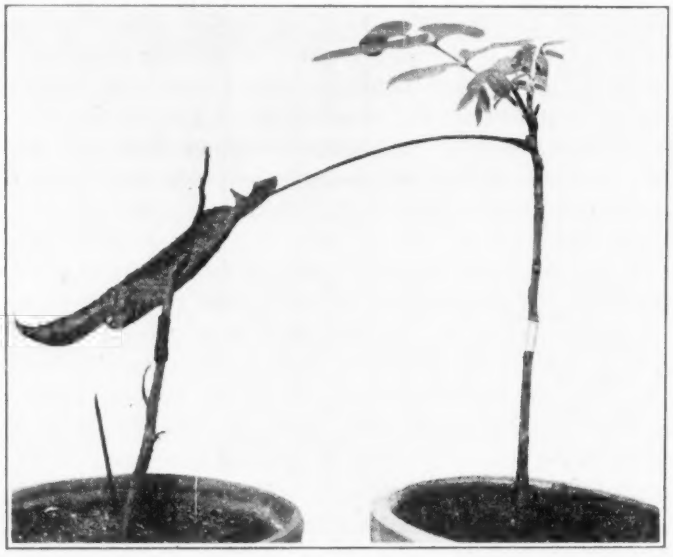

Fig. 5. Showing results of inoculation of seedling of *Juglans Sieboldiana* grown in good soil, but weakened by poor light and little water. Check plant at right. Spore pustules show on stem of inoculated plant at left. × about $\frac{1}{5}$.

found that under the callus a small, black area extended radially into the wood and thence had proceeded up and down the twig or branch a distance depending on the length of incubation. In both inoculated branches and twigs diseased from natural infections this black column, overlaid by healthy wood and bark, has been followed well into the trunk. In a small twig, it is conceivable that this fungal invasion, necessarily hindering the upward flow of liquids in the twig, would eventually result in so weakening it that the fungus will readily advance outward to cambium and bark—hence the death of the twig and sub-

sequent appearance of spore pustules. It is of course possible that the killing is due to the presence of some toxic substance secreted by the fungus or formed by the interaction of host and parasite, as Clayton (3) believes is operative in the tomato wilt. In the case of the larger twigs, naturally a longer time would be required for the lethal action of the fungus, whatever its *modus operandi*. In the trunk, in the case of a vigorous tree, the formation of the annual rings of wood goes on so rapidly as to cover up for a time the action of the fungus. Yet it may eventually reach a spot where small branches are given off, and advancing into these, slowly kills them. If the tree is weakened by drought or other unfavorable conditions such as fire (p. 427) the fungus advances more rapidly.

Sections of discolored wood have been stained, and show the presence of the fungous hyphae. The isolation of the fungus in culture media from such discolored wood has been extremely difficult and successful only in a few cases. Where segments of twigs with the discolored wood have been incubated in a moist chamber, they have invariably developed spore pustules. The case has already been described (p. 425) of the tree trunk inoculated in 1919, and cut in 1922, which showed the discolored wood covered by four layers of healthy wood, and developed the spore pustules after it had lain two months on the ground.

From the evidence presented in the foregoing it seems clear that we are dealing here with a weak parasite working slowly, or almost dormant, in vigorous trees—more rapidly in weak ones. After the lapse of many years it may gain the upper hand (Pl. XIX, figs. 1, 2) and the death of the tree is the result.

RELATIVE SUSCEPTIBILITY OF SPECIES OF JUGLANS

It will be seen by reference to the list of exsiccati and hosts that *Melanconis Juglandis* is by no means restricted to the butternut. Very probably the fungus may be found on any species of Juglans. But as far as our field observations go, pathogenic effects are most pronounced in *J. cinerea* and *J. Sieboldiana*, which is to be expected on account of the close relationship of those two species. In the experiments with healthy seedlings (p. 427), *J. Sieboldiana* proved extremely susceptible.

Although we have seen some trees of *J. nigra*, in the field, apparently seriously affected and showing all the symptoms of the trouble, we do not feel warranted at present, in view of the slight experience we have had with this species, in making any definite statement as regards its degree of susceptibility. But on account of its great economic importance

a thorough investigation is much needed. Likewise, regarding the susceptibility of *J. regia*, we know little.

As regards the western species of Juglans, we find no reference to a trouble like this. Thornber (20) states that the Arizona walnut, *Juglans major* Hell. does not appear to be troubled with any serious fungous disease. The walnut blight, so destructive to the Persian walnut, (*J. regia*) and its hybrids in California, is an entirely different disease. (18)

REMEDIAL MEASURES AND CONTROL

In the forest, any remedial or control measures are under present conditions impracticable. In view of the nature of this disease, as brought out by these studies, it is clear that trees with a large percentage of branches killed by Melanconis are in all likelihood trunk-infected. Their growth increment will necessarily be slow, the amount depending on the extent of infection. Such trees may as well be removed whenever practicable, as in improvement thinnings.

But in the case of orchard trees, or in parks and gardens, with proper care and vigilance the disease may be successfully combatted. In view of the fact that weakened trees in particular are attacked by the fungus, efforts should be made to maintain them in as healthy a condition as possible, by such sanitary measures as fertilizing, mulching, watering in dry seasons, pruning to increase vigor; and by avoidance of conditions which lead to winter injury, etc. The trees should be constantly watched, and all dead twigs pruned off as soon as discovered. It is hardly necessary to add that any twigs or branches showing fruiting bodies of Melanconis should be pruned off immediately, the cut being made some distance below the limit of the wood discoloration in the interior. All pruning wounds should be covered with paint or tar. After the fungus has penetrated the trunk, no remedy is practicable.[1]

SUMMARY

The evidence indicates that the Melanconis disease extends over the entire natural range of the butternut, and is one of its chief troubles; while the cultivated Japanese walnut (*J. Sieboldiana*) is likewise particularly susceptible. The disease is also of significance in its possible relations to other species of Juglans.

[1] Dr. Robert T. Morris states in correspondence, that "if blighted butternut trees are cut near the ground, and the stump sprouts grafted later with Persian walnut, black walnut, or heart nut scions, we are likely to obtain good crops from this species which are highly resistant to attacks from the Melanconium."

In trees which are previously weakened, the fungus makes rapid progress; otherwise the disease advances slowly. The evidence at hand does not appear to warrant the statement that there is danger of disappearance of the butternut.

In its first stages, the trouble is characterized by the appearance of dead limbs besprinkled with small black conidial acervuli. Occasionally these develop spore horns, but they are usually rounded or wartlike, and in wet weather like drops of thick ink.

This conidial stage has passed under the name of *Melanconium oblongum* Berk. The ascospore stage, which may appear later near these same pustules, has long been known as *Diaporthe Juglandis* E. & E. By culture work this is now definitely proved to be the perfect stage of *Melanconium oblongum* Berk. *Melanconis Juglandis* (E. & E.) comb. nov. is therefore proposed as the new name.

A pycnospore stage has appeared in cultures on both oat agar and peptone agar.

Inoculation experiments, extending over a period of more than four years, have demonstrated conclusively that the fungus is a weak parasite. Entering usually through small twigs by way of a wound, or through twigs already killed from some other cause, the mycelium grows slowly down through the wood, producing a black or dark discoloration, to the main branch and finally to the trunk. Thence any branch may become infected.

Ordinarily the progress of the disease is so slow that the leaves fall one by one, not producing any striking wilting or blighting effects. In final stages trees have a marked stagheaded aspect.

Diseased branches should be pruned off promptly some distance below the apparent infection, and the wounds tarred over or painted. After the fungus has penetrated the trunk, combative measures are useless.

For forest trees, no remedy is practicable under present conditions. Diseased trees should be removed during improvement thinnings.

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MELANCONIS JUGLANDIS ON BUTTERNUT

MELANCONIS JUGLANDIS ON BUTTERNUT

DESCRIPTION OF PLATES

PLATE XIX. MELANCONIS JUGLANDIS ON JUGLANS CINEREA

Figs. 1 and 2. A butternut tree at Hamden, Conn., showing the damage caused by the Melanconis disease in a two-year period. (1) Taken August 6, 1920; (2) Taken July 30, 1922. At A, B, and C are examples of branches which have died in the two-year interval. For detailed account see p. 424 of the text.

PLATE XX. MELANCONIS JUGLANDIS

Fig. 3. a, b, and c, successive stages in the development of the acervuli of the conidial stage of *Melanconis Juglandis* (E. & E.) comb. nov. on twigs of butternut: a, papillate swellings appearing; b, some of them burst open; c, warty, black masses of conidia. $\frac{4}{5}$ natural size.

Fig. 4. Acervuli, (the large black spots) on thick bark of trunk. $\frac{4}{5}$ natural size.

Fig. 5. Infected twig, incubated in a moist chamber, showing the extrusion of spores in horn-like masses from the acervuli. Such spore horns rarely occur in nature. $\frac{4}{5}$ natural size.

Fig. 6. Inoculated twig, showing where the inoculation was well healed over with a callus (about one inch from the base of photo); later, whole twig died as a result of growth of mycelium in interior, and numerous spore pustules appeared. This particular twig was a young green shoot at the time of inoculation. $\frac{2}{3}$ natural size.

Fig. 7. Part of old diseased twig showing holes in loose bark where spore pustules were located. $\frac{4}{5}$ natural size.

Fig. 8. Part of inoculated upright shoot of tree shown in Fig. 13, showing spore pustules in detail. Extent to which disease has apparently progressed is shown by irregular line below crotch. In the wood, however, the fungus has penetrated to the base of the tree, as was shown by subsequent examination. $\times$ 1.

Fig. 9. Branch of *Juglans Sieboldiana*, showing spore pustules appearing about the point of inoculation, some of them elongated or run together. About natural size.

Fig. 10. Ascogenous stage (not quite mature) appearing in cracks of butternut twig. $\times$ $1\frac{1}{5}$.

Fig. 11. Showing perithecial "necks" excessively developed, under moist chamber conditions. $\frac{4}{5}$ natural size.

Fig. 12. Pure culture on sterilized butternut twig, showing mycelial mounds where conidia will appear. About natural size.

Fig. 13. Seedling of *Juglans nigra* showing two inoculations, one of which has "taken," the fungus having progressed downwards. For further details see description of Fig. 8. About natural size.

THE PROBLEM OF BREEDING NEMATODE-RESISTANT PLANTS

WALTER SCOTT MALLOCH

WITH TWO FIGURES IN THE TEXT

The serious nature of nematode infestation has been pointed out by numerous writers. *Heterodera radicicola* is the most destructive form known at the present time. The theme of the present paper is to present some data collected in an attempt to breed plants resistant to *Heterodera radicicola*. The control of the root-knot nematode under greenhouse conditions is relatively simple compared to field conditions. E. A. Bessey (3) has reviewed the different methods for the control of the root-knot nematode under greenhouse and under field conditions. Although nematode infestation is serious enough under any condition, I shall take up the discussion of its control from the viewpoint of field conditions, which is by far the most difficult problem. A serious nematode infestation of a highly susceptible perennial plant is generally fatal. About the only treatment which can be safely given is to apply fertilizers and constant cultivation to induce the trees to grow faster than nematode galls can be produced. This method, however, cannot be regarded as highly satisfactory, due to its high cost and temporary nature. Perennial plants are stationary, and once the soil becomes infested, the plants are doomed, sooner or later. Even if the soil is free from root-knot nematodes at the time the trees are planted, no one can tell when root-knot nematodes will spread to this piece of land.

W. A. Orton (7) has stated that, "The root-knot nematode is already so widely distributed in California that it will be extremely difficult to restrict its further spread in any effective way by the application of quarantine measures." The same statement would apply to many other states in the Union. With perennial plants, crop rotation cannot be applied without great financial loss. One may say, then, that the breeding of nematode-resistant rootstocks for fruit trees is by far the most important of nematode-resistant problems. What little has been accomplished in this direction by the writer has been submitted for publication in another article.[1]

[1] Malloch, W. S. Asexual propagation as an aid to the breeding of rootstocks. (Submitted for publication.)

Let us now consider the problem of breeding for nematode resistance from the standpoint of annual plants. In breeding for disease resistance, the plant breeder has two alternatives: First, to find natural resistance in some variety or species and then to combine disease resistance or immunity with other desirable commercial characters. This is the most hopeful method from the breeder's standpoint. Second, it would be theoretically possible to create disease-resistant plants by transgressive segregation. This would imply that each parent possessed modifying factors which acting together would increase the resistance of the hybrid plants. Transgressive segregation does occur for certain characters such as tetramerous flowers in tobacco. Some varieties of tobacco have a reduced number of parts of one or more floral whorls. *Nicotiana Tabacum* var. *Havanensis* was found to produce on the average 18.814 ± 0.203 per cent tetramerous or otherwise reduced flowers on the same plant with the normal pentamerous flowers.[1] The extremes for this variety being 14 to 26 per cent. *Nicotiana Tabacum* var. *purpurea* on the other hand does not produce any tetramerous or otherwise reduced flowers. The F_1 hybrid between these two parents produced, on the average, 1.127 ± 0.792 per cent tetramerous or otherwise reduced flowers per plant. The F_2 produced all the way from 0.0 to 54.0 per cent tetramerous or otherwise reduced flowers per plant, thus exceeding either parent in the percentage of reduced flowers on any particular plant. In this case, the factor or factors for tetramerous flowers was clearly present in one parent. If a certain amount of resistance could be demonstrated in a particular variety, then we might hope to increase this resistance by hybridization.

Theoretically, the finding of resistance in one parent should not limit the building up of resistant forms. In the inheritance of aleurone color in corn, East and Hayes (5) found that two white varieties might each possess factors all of which are necessary for the production of color. A white variety of the genetic constitution CCrrppii crossed with another white variety carrying the factors ccRRppii gives a red type in F_1 and 9 red to 7 white in F_2. I am not aware of any demonstrated case of this type in the inheritance of disease resistance. As a matter of fact, the reverse has been demonstrated. O. S. Aamodt (1) found in a cross between Kanred and Marquis wheats that all the F_2 plants were susceptible to biologic forms of *Puccinia graminis tritici*, to which both parents were susceptible. The controlled tomato hybrids made by the writer were all susceptible to nematode attack.

[1] Malloch, W. S. Studies of inheritance in tobacco and maize. Master's Thesis. 1920. (Manuscript.) University of California Library, Berkeley, California.

In breeding for disease resistance, it is frequently found that resistance is only obtained by crossing with resistant species. Beach and Maney (2) found that *Prunus besseyi* (Sand cherry) is immune to aphis attack, while *Prunus cerasus* (Montmorency cherry) and *Prunus Americana* (Wyant plum) are susceptible to aphis attack. These species cross freely and give a ratio of about 3 immune to 1 susceptible form in F_2. But there are many species, like *Hordeum vulgare* and *Hordeum murinum*, which do not cross easily and in which the resulting hybrids do not reach maturity (6). In such cases as these, the knowledge gained by geneticists should be of great service to plant breeders.

EXPERIMENTAL RESULTS

In the description of the results of the present experiment, I have included a brief discussion of cultural methods for growing plants and nematodes together. This portion has been contributed for what little value it might be to further studies on the causes of immunity. A few more observations have been made on cross inoculation tests which serve to confirm the opinion that there are no physiological or biological strains of *Heterodera radicicola*. This is a distinct advantage to the breeding of nematode-resistant plants, for once a resistant plant is found, it should be resistant to any new infestation of *H. radicicola*. Such a condition is a disadvantage in crop rotation, however, as the same form of *H. radicicola* will attack many of the crops which would be desirable for rotation purposes. Finally, I have included a list of the species and varieties of plants found to be susceptible to *H. radicicola*.

CULTURAL METHODS OF GROWING PLANTS FOR STUDYING IMMUNITY TO NEMATODES

In order to study immunity of different varieties and species to nematodes and the causes for any immunity found, we must have some method of growing the plants and nematodes together. The natural method of infection from nematodes is from larvae present in the soil. Soil cultures are the most desirable for studying the relative resistance of different plants, but are unsuited for studying certain suspected causes of immunity, such as differences in anatomical structure. In the present investigation, a greenhouse soil badly infected with nematodes from Gardenia plants was used in the greenhouse tests. It was planned to test relative degrees of resistance by growing plants in the same type of soil which had been hauled to an isolated piece of land in Berkeley, but it was necessary to terminate the experiment before this could be accomplished.

For anatomical work, it is desirable to grow the plants in a culture medium, where the roots will be free from adhering soil particles. This may be accomplished by allowing the roots to grow through the drainage hole in a flower pot in which the plant is growing. It may also be accomplished by growing the plant in a nutrient solution. For chemical studies, it is often desirable to be able to control the culture media in which the plants are grown.

L. B. Byars (4) found that plants grown in Pfeffer's nutrient agar became infected when nematodes were added to the culture.

Pfeffer's Nutrient Agar

Calcium nitrate	$CaNO_3$	4.0 grams.
Potassium nitrate	KNO_3	1.0 "
Magnesium sulphate	$MgSO_4$	1.0 "
Potassium dihydrogen phosphate	KH_2PO_4	1.0 "
Potassium chloride	KCl	0.5 "
Distilled water	H_2O	6.0 liters.
Powdered agar		12.0 grams.

The writer found that Dwarf Champion (No. 13), Earliana (No. 73), and Matchum (No. 84) tomatoes and Whippoorwill (No. 2) cowpea became infected when nematode galls were added to the culture. The agar method has an objection in that it soon becomes infected with other organisms as observed by Byars (4), and Steiner and Heinly (8). The writer found that the agar became infected even when nematodes were not added to the culture media. Infection probably takes place when fresh agar is added to the bottle in which the plants are growing. In the present experiment, the details as to sterilization and preparation of the nutrient agar described by Byars (4) were followed out. At first the bottles were only filled one-third full with Pfeffer's nutrient agar. Sterilized seeds were sown upon the nutrient agar and the cotton plugs replaced. Most of the seedlings had a tendency to sink and become immersed in the fluid. Those which did not have this difficulty failed to grow more than about three inches, due probably to lack of air (Fig. 1).

To avoid this difficulty, tests were conducted with cowpeas which were sown upon the top of supports in the mouths of bottles. The bottle was filled with Pfeffer's nutrient agar until the sterilized seeds were partially immersed in the fluid. Two types of supports were used. An inverted strip of wire screening, one inch wide and six inches long, served for one type. Sterilized seeds were sown upon this wire support. The mouth of the bottle was then stoppered with a cotton plug. When the plants had germinated, the cotton was placed around the stem in such a manner as not to interfere with its growth above the bottle. A

piece of cheesecloth thinly covered with cotton and pressed down into the mouth of the bottle served as a second type of mechanical support. Sterilized cowpea seed was sown upon this support, which was in contact with the agar medium. In both methods, the plants developed normally to a height of about one foot (Fig. 2). Infection also developed in this method.

To avoid contamination from other organisms, an attempt was made to devise media in which both nematodes and plants could develop but in which other organisms could not. Nematodes were added to pure silica sand in a one-half pint milk bottle. Seeds from different plants were then sown in this bottle and the plants kept alive by frequent

FIG. 1. Cantaloupe seedlings growing on Pfeffer's nutrient agar. The seedling in the bottle on the right has become immersed in the fluid.

additions of Pfeffer's nutrient solution. This solution has the same composition as Pfeffer's nutrient agar minus the agar. Whippoorwill cowpea (No. 2) and Red Currant tomato (No. 63) became infected in this culture medium. Such a method might be of value for certain chemical investigations but would be little better than a soil culture for anatomical studies.

Whippoorwill cowpea (No. 2) plants, five inches high, and Orobia (No. 76), Red Cherry (No. 81), and Striped (No. 78) tomato plants, one to one and a half feet high, which had been previously grown in nematode-free soil, were transplanted into Erlenmeyer flasks. Pfeffer's nutrient solution was maintained at about one-half inch depth in the flasks in which the plants were placed. The bottom of the flask was

about six inches wide, giving plenty of room for root development. The plants were grown in this way from Nov. 1, 1922, to Jan. 18, 1923. All of the varieties became infected and showed the presence of numerous galls on the roots. Algae developed both in the sand cultures and in the water cultures. The growth of algae may be suppressed, however, by keeping the culture media in the dark and only exposing the tops of the plants to the light. The water-culture method should be well suited for anatomical studies.

FIG. 2. Cowpea seedling growing on Pfeffer's nutrient agar. The one on the left is supported by wire screening. The one on the right is supported by a cheesecloth-and-cotton support.

CROSS-INOCULATION EXPERIMENTS

E. A. Bessey (3) has discussed the possibility of *Heterodera radicicola* developing physiological strains which prefer certain hosts and exhibit a reluctance to attack other hosts. Such strains are known in *Tylenchus dipsaci* and other organisms, and these strains are morphologically indistinguishable from one another. Carefully washed roots of a plant infected with *Heterodera radicicola* were placed in a pot of sterilized soil. Seeds of another kind of plant were sown in this pot, to determine if they became infected with nematodes of the other plant. Experiments

made in this way showed that *Heterodera radicicola* were mutually interchangeable in the following plants: Red clover (*Trifolium pratense*), white clover (*T. repens*), crimson clover (*T. incarnatum*), cowpea (*Vigna unguiculata*), strawberry (*Fragaria chiloensis*), tree morning-glory (*Ipomoea syringaefolia*), sunflower (*Helianthus debilis*), horse bean (*Vicia faba*), ginseng (*Panax quinquefolium*), purslane (*Portulaca oleracea*), fig (*Ficus carica*), papaya (*Carica papaya*), catalpa (*Catalpa speciosa*), tomato (*Lycopersicon esculentum*), and Old World grape (*Vitis vinifera*). All these affect the following, for which the reverse inoculation experiments were not made: Lettuce (*Latuca sativa*), green gram (*Phaseolus radiatus*), tobacco (*Nicotiana tabacum*), squash (*Cucurbita moschata*), cucumber (*Cucumis sativus*), and muskmelon (*C. melo*).

Bessey concludes that, "The various families of plants represented in the foregoing list and the fact that the infections were obtained easily and very pronouncedly would seem to indicate the nematode causing root-knot of the plants experimented with, including some of those most generally affected in the field, is not as yet very markedly differentiated into strains peculiar to certain hosts." The results of the present experiment, so far as they go, seem to confirm this view. A large number of sources of *Heterodera radicicola* were not available, but the different sources which were available were utilized. The experiment has not progressed far enough to develop new sources. The results of cross-inoculation experiments are summarized in table 1. Infection took place readily in each case reported. The culture media mentioned in the table are described in another paragraph of the article.

TABLE 1—*Results of cross-inoculation experiments.*

Plant under test	Source of nematodes	Culture medium	Where grown
Cantaloupe varieties	Gardenia plants	Soil	Greenhouse
Whippoorwill cowpea	Sesbania "	Pfeffer's Nutrient Solution.	Laboratory
" "	Tomato plants	Pfeffer's Nutrient Agar	Laboratory
" "	" "	Sand	Laboratory
Tomato varieties	Gardenia plants	Soil	Greenhouse
Striped tomato	" "	Pfeffer's Nutrient Solution	Laboratory
Red Cherry tomato	" "	Pfeffer's Nutrient Solution	"
Red Currant "	" "	Sand	"

Infection took place in each case. The sand cultures were supplied with Pfeffer's Nutrient Solution. See tables 3 and 4 for cantaloupe and tomato varieties.

PLANTS FOUND TO BE INFECTED BY HETERODERA RADICICOLA

A number of different plants were sent to the writer for examination during the year. I have included all of those which showed positive infection in table 2. Some of these have been reported by other writers

TABLE 2—*Plants affected by Heterodera radicicola.*

Name	Locality obtained from
Begonia sp.	Greenhouse Berkeley, California
Gardenia sp.	" " "
**Lotus corniculatus*	Field in Imperial Valley, Calif.
Medicago hispida reticulata	" " " " "
**Phaseolus max* (Mung bean)	" " " " "
" " " "	Field in Fresno Co., California
**Pisum sativum*	Field in Imperial Valley, Calif.
Prunus persica	Berkeley, Calif.
Sesbania macrocarpa	Field in Imperial Valley, Calif.
Soja max (Mammoth Yellow Soybean)	" " " " "
" " (Ito San Soybean)	" " " " "
" " (Virginia Soybean)	" " " " "
" " (" ")	Field in Fresno Co., California
**Stizolobium deeringianum* (Velvet bean)	" " " " "
**Trifolium alexandrium*	Field in Imperial Valley, Calif.
" " *Muscowi*	" " " " "
" " *Saidi*	" " " " "
**Trignonella foenum graceum*	" " " " "
Vicia agritinense	" " " " "
V. biennis	" " " " "
V. dasycarpa	" " " " "
**V. faba*	" " " " "
**V. fulgens*	" " " " "
V. hybrida	" " " " "
V. ludoviciana	" " " " "
V. pannonica	" " " " "
Vicia peregrina	" " " " "
V. polyphylla	" " " " "
**V. sativa dura*	" " " " "
" " *leucosperma*	" " " " "
V. stricta	" " " " "
V. villosa	" " " " "
Vigna unguiculata (Blackeye)	" " " " "
" " (Brabham)	Berkeley and Imperial Valley
" " (Iron)	" " " "
" " (New Era)	Fresno and Imperial Valley
" " (Whippoorwill)	Berkeley and Imperial Valley

but have been included as confirmatory evidence. In every case, living organisms were found besides the characteristic nematode galls or swellings on the roots. Plants which were not infected have not been listed, because we have no way of judging as to immunity unless the plants have been grown under controlled conditions.

VARIETIES OF CANTALOUPES AND TOMATOES FOUND TO BE SUSCEPTIBLE TO HETERODERA RADICICOLA

Sixty-seven distinct varieties of cantaloupes were found to be susceptible to nematode attack. Ninety-eight separate varieties and eight controlled hybrids of tomatoes were also found to be susceptible. In tables 3 and 4, I have listed the names of these varieties and the localities where they were obtained. The varieties were obtained from widely separated localities, as shown in the table. In many instances, samples of a given variety, like Earliana, were obtained from several different localities. Some samples were obtained from one party who had in turn received them from several different sources. In the case of nematode infection, the locality from which the variety was obtained had no effect on resistance, since no biological strains of *Heterodera radicicola* are known. All of the varieties and all of the samples of these varieties of cantaloupes and tomatoes listed in tables 3 and 4 were found to be susceptible to *Heterodera radicicola*.

In order to measure relative degrees of resistance, the plants must be grown under normal conditions, where they can complete their normal life cycle. The plants in the present experiment were largely grown in pots under greenhouse conditions, although a few varieties were grown under field conditions, and a few varieties were grown in the laboratory. In every case, however, the test was of value for estimating immunity or susceptibility rather than relative degrees of resistance. The section on Cross-Inoculation Experiments lists the sources of nematodes for the greenhouse and laboratory experiments. A few tomato varieties were grown on an infected piece of land at the U. S. Plant Introduction Gardens at Chico, Calif., but frost killed the plants before they completed their normal life cycle. Observations were made on the Pollock 10–25 cantaloupe in the Imperial Valley, but no data can be given on resistance, due to lack of controlled conditions. For this reason, the detailed data for each variety has not been presented. The essential fact of the test is that every variety examined is at least susceptible to the attack of *Heterodera radicicola*.

TABLE 3—*Varieties of cantaloupes found to be susceptible to Heterodera radicicola.*

Variety	Culture no.	Locality obtained from
Unnamed	48	Pullman, Washington
"	49	" "
"	51	" "
Acme	9	San Francisco, California
Admiral Togo	60	Detroit, Michigan
Angella	47	Twin Falls, Idaho
Armenian	20	Los Angeles, California
Banana	23	" " "
Bay View	6	San Francisco, California
Blenheim Orange	54	Philadelphia, Pennsylvania
" "	78	Reading, England
Burrell's Gem	8	San Francisco, California
California Beauty	22	Los Angeles, California
Casaba Mixture	19	" " "
" "	25	" " "
Champion Market	65	Detroit, Michigan
Defender	63	" "
Egyptian Deity	35	College Park, Maryland
Emerald Gem	11	San Francisco, California
Empress	80	Reading, England
Extra Early Hackensack	13	San Francisco, California
Extra Tip Top	75	Indianapolis, Indiana
Fordhook	56	Philadelphia, Pennsylvania
Gautier's Pineapple	29	Los Angeles, California
Gold Lined Rocky Ford	14	San Francisco, California
Golden Beauty Casaba	4	San Francisco, California
Golden Eagle	58	Philadelphia, Pennsylvania
Hackensack	10	San Francisco, California
Half Hardy Green Fleshed	81	Reading, England
Half Hardy Scarlet Fleshed	82	" "
Honey Dew	42	Urbana, Illinois
" "	67	Lexington, Kentucky
Honey Dew, Hybrid Casaba	16	San Francisco, California
Hoodoo	44	Urbana, Illinois
"	64	Detroit, Michigan
Improved Hybrid Casaba	27	Los Angeles, California
Indiana Cantaloupe	33	College Park, Maryland
Jenny Lind	21	Los Angeles, California
" "	43	Urbana, Illinois
" "	62	Detroit, Michigan
King George	85	Reading, England
Large Hackensack	26	Los Angeles, California
Maryland Gem	74	College Park, Maryland
Maryland Genus	32	" " "
Mixed Cantaloupe	86	Reading, England
Montreal Green Nutmeg	38	Urbana, Illinois
" " "	69	Lexington, Kentucky

TABLE 3—*Continued*

Variety	Culture no.	Locality obtained from
Montreal Market	15	San Francisco, California
Montreal Market Nutmeg	41	Urbana, Illinois
Netted Rock	30	Los Angeles, California
Osage	24	Macdonald College, Quebec
Osage Gem	17	Los Angeles, California
Paul Rose	18	Los Angeles, California
" "	61	Detroit, Michigan
Persian	2	San Francisco, California
Pollock 10–25	7	" " "
Pollock 10–25 Selections	1	Imperial Valley, California
Rocky Ford	3	San Francisco, California
" "	46	College Park, Maryland
" "	68	Lexington, Kentucky
Rosa	71	Waverly Mills, South Carolina
Royal Jubilee	91	Reading, England
Salmon Tint Pollock–25	57	Philadelphia, Pennsylvania
Santa Claus	28	Los Angeles, California
" "	37	College Park, Maryland
Scarlet	92	Reading, England
Selection of Buskuks Gem	34	College Park, Maryland
Spicy	59	Philadelphia, Pennsylvania
Superlative	93	Reading, England
Texas Camionball	70	Lexington, Kentucky
Ten Twenty Five	39	Urbana, Illinois
Tip Top	12	San Francisco, California
" "	40	Urbana, Illinois
Tait's Thoroughbred Bottomly Muskmelon	52	Norfolk, Virginia
Thoroughbred Knight Muskmelon	53	" "
Universal	95	Reading, England
Vaughan's Original Osage	45	Urbana, Illinois
Windsor Castle	55	Philadelphia, Pennsylvania
Winter Pineapple	5	San Francisco, California

TABLE 4—*Varieties of tomatoes found to be susceptible to Heterodera radicicola*

Variety	Culture no.	Locality obtained from
Acme	266	Moscow, Idaho
Adirondack Earliana	267	" "
Albino	305	Lincoln, Nebraska
"	306	" "
Anne	145	Berkeley, California
Atlantic Prize	23	Los Angeles, California
" "	268	Moscow, Idaho

TABLE 4—*Continued*

Variety	Culture no.	Locality obtained from
Baur's Yellow	30	College Park, Maryland
" "	34	" " "
B.C. 2–A	50	" " "
" "	51	" " "
Beauty	15	San Francisco, California
Best of All	303	Reading, England
Bonnie Best	12	San Francisco, California
" "	26	Durham, New Hampshire
California Wild	125	Berkeley, California
Canner	48	College Park, Maryland
Chalk's Early Jewel	14	San Francisco, California
Cherry	46	College Park, Maryland
Coreless	53	" " "
"	169	Farmington, Utah
Cuke × Coreless Borghese	45	College Park, Maryland
Dwarf Aristocrat × Buckeye State	39	" " "
" " " "	49	" " "
Dwarf Champion	13	San Francisco, California
" "	260	Waverly Mills, South Carolina
" "	270	Moscow, Idaho
Dwarf Giant	271	Moscow, Idaho
Dwarf Ponderosa	272	" "
Dwarf Quarter Century	176	Farmington, Utah
Dwarf Stone	131	Philadelphia, Pennsylvania
" "	177	Farmington, Utah
Eailibell A	273	Moscow, Idaho
Eailibell B	274	" "
Earliana	16	San Francisco, California
"	73	State College, Pennsylvania
"	146	Berkeley, California
"	275	Moscow, Idaho
"	276	Philadelphia, Pennsylvania
"	277	Moscow, Idaho
"	278	New York, N. Y.
"	279	Moscow, Idaho
"	280	" "
"	281	" "
"	282	" "
"	283	New York, N. Y.
Earliest Market	284	Moscow, Idaho
Early Detroit	11	San Francisco, California
Early June	286	Moscow, Idaho
Early Market	285	" "
Early Prolific	287	" "
Early Ruby	288	" "
Fiascove	83	Mexico by U. S. D. A.

TABLE 4—*Continued*

Variety	Culture no.	Locality obtained from
Ficarazzi	264	Columbia, Missouri
Fifty Day	289	Moscow, Idaho
First Early	22	Los Angeles, California
Fusarium Wilt Resistant	29	Georgia
Globe	28	"
Golden Dwarf Champion	290	Moscow, Idaho
Golden Queen	9	San Francisco, California
" "	77	Santa Ana, California
Hardy Jewel	147	Berkeley, California
Hummer	32	College Park, Maryland
Individual Coreless Borghese	54	" " "
John Baer	5	San Francisco, California
" "	291	Moscow Idaho
June Pink	292	" "
Kansas Standard	293	" "
Large Meaty	40	College Park, Maryland
Large Pear	44	" " "
Large Plum	52	" " "
Large Yellow	238	Reading, England
Livingston's Coreless	20	Los Angeles, California
Livingston's Favorite	24	" " "
Livingston's Globe	8	San Francisco, California
" "	72	Columbia, Missouri
Louisiana Pink	261	Davis, California
Maryland Slicing	31	College Park, Maryland
" "	37	" " "
Maryland Solid	27	" " "
Matchless	6	San Francisco, California
Matchum	84	State College, Pennsylvania
Missouri 307	254	Columbia, Missouri
" 309	255	" "
" 313	256	" "
" 314	257	" "
" 317	258	" "
Moore's Early	294	Moscow, Idaho
Murray's New Perfection	36	College Park, Maryland
Narduke	57	Lexington, Kentucky
Norton	56	" "
"	88	Santa Ana, California
"	140	" " "
"	205	Farmington, Utah
Orobia	76	Mexico through U. S. D. A.
"	265	Columbia, Missouri
Payne's Victory	25	Los Angeles, California
Peach	21	" " "

TABLE 4—*Continued*

Variety	Culture no.	Locality obtained from
Pierrette	307	Paris, France
P-L-M	308	" "
Ponderosa	7	San Francisco, California
"	108	Manhattan, Kansas
"	143	Philadelphia, Pennsylvania
Porto Rico Wild × (Cherry × Maryland Solid)	47	College Park, Maryland
Porto Rico Wild × (Cherry × Red Rock)	35	" " "
Precore	82	Mexico through U. S. D. A.
Princess of Wales	304	Reading, England
Red Cherry	81	Santa Ana, California
Red Currant	63	Santa Ana, California
Red Forty Day Genoa	263	Columbia, Missouri
Red Head	295	Moscow, Idaho
Red Pear	1	San Francisco, California
" "	65	Santa Ana, California
" "	139	San Francisco, California
Red Riding Hood	297	Moscow, Idaho
" " "	298	" "
" " "	299	" "
Red Rock	19	Los Angeles, California
" "	296	Moscow, Idaho
Reich × Coreless	33	College Park, Maryland
Rosso	75	Mexico through U. S. D. A.
San José Canner	4	San Francisco, California
Selection	41	College Park, Maryland
Self Pruning	300	Moscow, Idaho
Stone	17	San Francisco, California
Striped Tomato	78	Berkeley, California
Sunrise	269	Moscow, Idaho
Tister's Empire	55	College Park, Maryland
Trophy	18	San Francisco, California
Victoria Whole Salid	301	Moscow, Idaho
Yellow Cherry	86	Santa Ana, California
Yellow Cherry	302	Moscow, Idaho
Yellow Pear	2	San Francisco, California
" "	64	Santa Ana, California
" "	138	San Francisco, California
Yellow Plum	3	" " "
" "	137	" " "
" "	142	Santa Ana, California
Yellow Ponderosa	66	" " "
Yep	69	Macdonald College, Quebec
"	148	" " "

TABLE 5—*Controlled hybrid tomatoes found to be susceptible to Heterodera radicicola.*

Red Currant	22–63 P–21 × Red Cherry	22–81 P–20	22.3 F_1–6
Yellow Cherry	22–86 P–8 × " "	22–81 P–8	22.3 F_1–2
" "	22–86 P–8 × Red Currant	22–63 P–21	22.3 F_1–1
Yellow Pear	22–64 P–30 × Earliana	22–70 P–8	22.3 F_1–13
" "	22.8–2 P–1 × Red Cherry	22–81 P–20	22.3 F_1–11
" "	22.8–2 P–2 × Red Currant	22–63 P–21	22.3 F_1–9
" "	22.8–2 P–1 × Yellow Plum	22.8–3 P–1	22.3 F_1–10
Yellow Plum	22–62 P–31 × Red Currant	22–63 P–21	22.3 F_1–12

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A SPECIES OF OLPIDIUM PARASITIC IN THE ROOTS OF TOMATO, TOBACCO AND CABBAGE

MATHILDE BENSAUDE

WITH FIVE FIGURES IN THE TEXT

Woronin[1] in 1878 found a parasitic fungus in the roots and hypocotyl of blighted cabbage seedlings and named it *Chytridium brassicae*. The organism was later transferred to the genus Olpidium by Dangeard,[2]

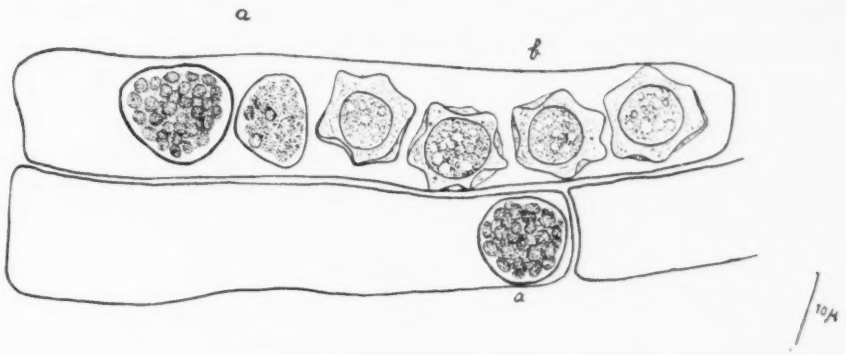

FIG. 1. Cross-section through cortical cells of tomato root containing zoosporangia (a) and resting spores (b) of *Olpidium sp.* (Camera lucida drawing, host cells semi-diagrammatic.)

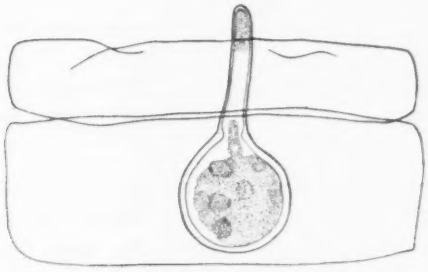

FIG. 2. Cross-section of zoosporangium with neck-like structure having reached the surface of the parasitized cabbage root. The protoplasm is dividing into zoospores. (Camera lucida drawing, the host cells semi-diagrammatic.)

and is now known as *Olpidium brassicae* (Wor.) Dangeard. The pathogenicity of the fungus has been assumed without any experimental

[1] Woronin, Jahrb. Wiss. Bot. **11**: 556. 1878.
[2] Dangeard, Ann. Scienc. Nat. Ser. 7, **4**: 327. 1886.

evidence. Peters and Schwarz,[1] in a bulletin on tobacco diseases, report that a yellowing and blighting of tobacco seedlings caused by *Olpidium brassicae* has often been observed in Dalmatia and Gallizia. Root cells of tobacco invaded by Olpidium are figured after Preissecker. The writer has not been able to find in the bulletin or anywhere else the reference to Preissecker's work, and has not seen the original paper.

Since the writer has found no case of Olpidium, as a root parasite, reported in American literature, it seems worth while to give a brief description of an Olpidium infection of the roots of several plants observed in the green house while working with another root disease of tomato and tobacco at the University of Wisconsin.

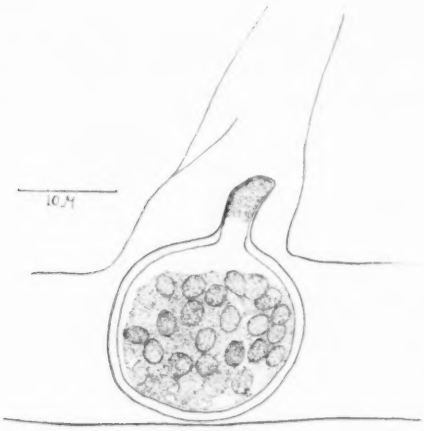

FIG. 3. Cross-section through a zoosporangium parasitic in a cabbage root cell. The neck is growing into a root hair. (Camera lucida drawing, the host cell semi-diagrammatic.)

The infection was first found on tomatoes. Tobacco and cabbage plants grown in the same soil became readily parasitized. Infected plants developed quite normally. Even the roots looked healthy save for some areas of superficial and very slight discoloration. Seeds of cabbage and tobacco sown in infected soil produced quite normal seedlings, yet upon examination the roots proved to be heavily infected. In its morphology the fungus found in roots of tomato, tobacco, and cabbage corresponds very closely to the description of *Olpidium brassicae* given by Woronin. The zoosporangia are found singly or several together in cortical cells. They are most abundant in the outer layer,

[1] Peters and Schwarz, Mitt. K. Biol. Anst. f. Land- u. Forstwirtsch. Heft **13**:10. 1912.

but are also often found at a considerable distance from the surface, as far inward as the fourth or fifth row of cells. These bodies are spherical or else slightly elongated in the direction of the longer axis of the host cell. The walls are smooth, relatively thin, and very permeable to stains. The protoplasm, dense in young sporangia, divides when maturity approaches into areas corresponding to the future zoospores. At maturity the zoosporangia produce a neck-like structure through which the zoospores are liberated. In our preparations a plug of dense protoplasm was always present at the apex of these structures before

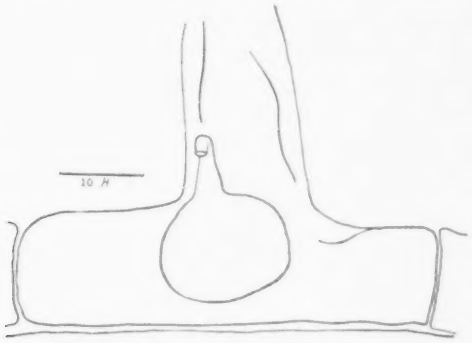

FIG. 4. Zoosporangium having discharged the zoospores, apparently into a root hair. (Outline of zoosporangium drawn with camera lucida, host cells semi-diagrammatic.)

FIG. 5. Surface view of resting spore teased out of host cell.

rupture. The latter vary greatly in length from one zoosporangium to another. Most of them grow through one or several cells to the surface of the host (Fig. 2) and apparently discharge their contents directly into the soil. Others never reach the surface of the host and probably liberate the zoospores into the cortical cells or root hairs in which they terminate (Figs. 3 and 4). According to Woronin the zoospores of *Olpidium brassicae* are spherical, with one single anterior flagellum. Having never seen mature zoospores we do not know if this is also the case for the Olpidium in question. Resting spores similar to those

described by Woronin were found abundantly in the outer cell layer of roots, often at the base of root hairs. The resting spores are white or light yellow with a thick wall impenetrable to thionin; the protoplasm is colorless, granular and rich in oil droplets. Young resting spores are slightly polygonal in contour, older ones become wrinkled and covered with ridges (Fig. 4), appearing star-shaped in cross section (Fig. 1). Characteristic thick-walled resting spores were observed not exceeding 6 to 7 microns in diameter. The larger ones found attained a diameter of 30 microns. The ultimate development of the resting spores has not been observed.

Judging by the figures and short description of Woronin, the Olpidium described in this note seems to be identical with *Olpidium brassicae* (Wor.) Dangeard. The serious pathogenic action attributed to this fungus by Woronin and other above mentioned authors was not observed. Further investigation would be necessary in order to ascertain whether the earlier writers were justified in attributing to the Olpidium present in the lesions the blighted condition of cabbage and tobacco seedlings, and whether the Olpidium observed in Wisconsin is really identical in every respect to the European *Olpidium brassicae* (Wor). Dangeard.

UNIVERSITY OF WISCONSIN.

A NEW HOST FOR BACTERIUM MALVACEARUM

J. G. Brown and Frederick Gibson

With Two Figures in the Text

In the autumn of 1922 it occurred to the writers that cross-inoculations of Pima-Egyptian cotton and Arizona wild cotton (*Thurberia thespesioides*), with the angular leaf spot organism, *Bacterium malvacearum*,

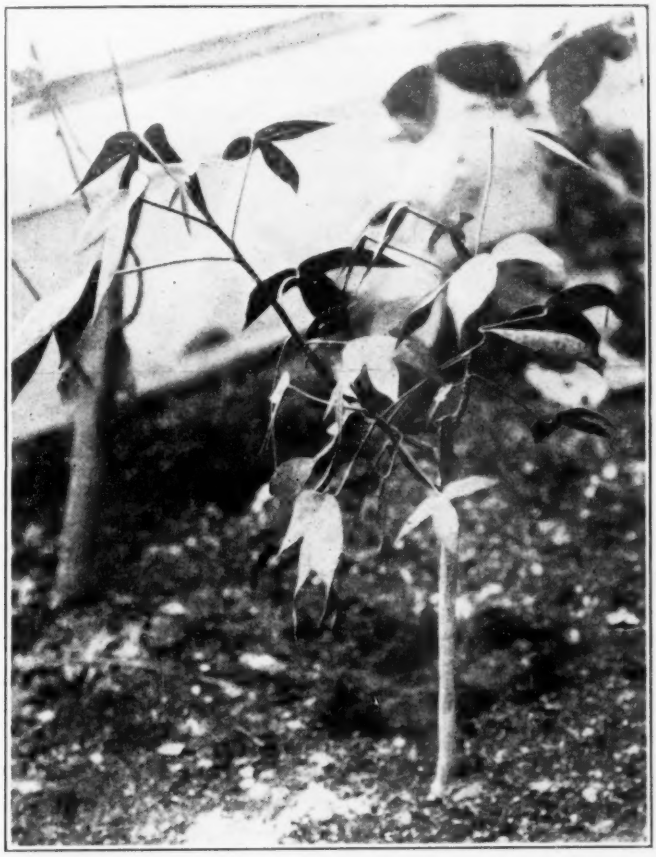

Fig. 1. Young plant of Arizona wild cotton, *Thurberia thespesioides*.

should prove interesting. Accordingly seeds of Thurberia were planted in a bed in the greenhouse, which already contained plants of the Pima-Egyptian strain of Gossypium, and inoculations were later made which are briefly described below.

Thurberia thespesioides is a perennial shrub varying in height from four or five feet to ten feet or more. It grows in washes, arroyos, canyons, and on caynon walls in southeastern Arizona, at altitudes of two thousand to seven thousand feet. The leaves, which are mostly tri-lobed, are thinner and more delicate and the stems woodier than those of cultivated varieties of Gossypium.

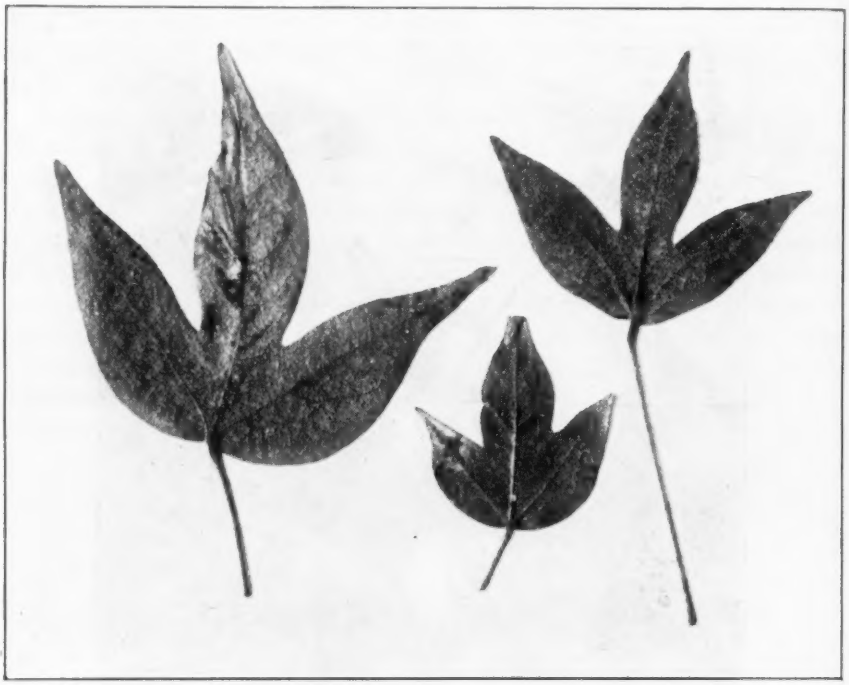

FIG. 2. Leaves of Arizona wild cotton showing spots infected with the angular leafspot organism *Bacterium malvacearum*. The leaf on the left has a dying middle lobe; that on the right has a marginal infection.

When the plants of Thurberia in the greenhouse were about one foot high they were inoculated with a pure culture of *Bacterium malvacearum* obtained from Pima-Egyptian cotton grown on the Salt River Valley Farm of the Arizona Agricultural Experiment Station. Inoculations were made by spraying and also by rubbing the inoculum on the leaf surfaces with the fingers. At the same time the Pima-Egyptian plants were treated similarly. Infections resulted in both kinds of plants, although they were slower to appear in the Thurberia plants. From the infected areas, the angular leaf spot organism was isolated.

The infected spots on the wild cotton were generally confined to the leaves and were usually found along the veins as shown in figure 1, whether the leaves were young or mature. In a few leaves the infected areas were marginal, and in some others they were intervenar and exactly similar to the angular spots on cultivated cotton caused by *Bacterium malvacearum* except that they were smaller because of the smaller size of the intervenar spaces in leaves of Thurberia. Eventually the infected area became dark brown, the tissues yellowed beyond the diseased region, and the leaf dropped. Shedding of the leaves of Thurberia appears to result from much less infection than is the case with cultivated varieties of true cotton. Only one typical black arm lesion was found, which was located on a green twig.

ARIZONA AGRICULTURAL EXPERIMENT STATION,
TUCSON, ARIZ.

DISCOVERY OF CURLY LEAF OF SUGAR BEETS IN THE ARGENTINE REPUBLIC

P. A. Boncquet

During the season from August 1916 till February 1917, the writer was investigating the possibilities of the sugar beet industry in the Argentine Republic. This study was started under the direction of the Franklin & Herrera Land Company of Buenos Aires. Three stations were established: one in San Isidro, one in Guatrache and one in Colonia Alvear. Small plots of beets were grown and the development of the beet noticed.

The curly leaf of beets was first observed at San Isidro, a suburb of Buenos Aires. The plants affected were a variety of small beet growing spontaneously along the Rio de la Plata. The black rings in the root, the protuberances on the leaves, and the dwarfing of the heart leaves were characteristic signs of the presence of the disease. The discovery, however, was so unusual and so unexpected, that it did not convince the writer to his complete satisfaction. A search for more evidence was at once undertaken and a great number of vegetable gardens around Buenos Aires, where garden beets were grown, were visited. In most places, where garden beets were found, the same symptoms of the disease were observed. But, as it was winter then, the disease was rather of a mild type. Efforts were made to find specimens of *Eutettix tenella* Baker, the leaf hopper whose sting incites curly leaf. A few were captured and examined. They had the same appearance as those which the writer captured in Berkeley, California, in 1914. They were dark grey, full-grown and ready for oviposition. Dark specimens of the same kind have repeatedly been observed during the winter of 1915 in the Salinas Valley, California. There, they were hiding under the grass in the irrigation ditches and on sunny days became active on those sides of the ditches which were exposed to the sun. This winter form of the insect was observed not only in the beet gardens, but also along the Rio de la Plata at San Isidro, the seasonal and regional conditions being very similar to those in Berkeley and in the Salinas Valley in California.

Upon his arrival in Colonia Alvear (Western Argentina), the writer visited the experimental beet plots of the Colonia Alvear Land Company. The beets which had been left from the previous year had all gone to seed. However, the signs of curly leaf were evident. The protuberances on the leaves and the rings in the roots, characteristic of the

disease, were noted. Microscopic examination revealed also the characteristic ring-shaped and S-shaped bodies in the necrotic phloem bundles.

By this time, hardly any doubt could remain as to the presence of the disease in the southern republic. A few specimens of *Eutettix tenella* were also discovered there at this time. Nevertheless, a careful study was undertaken in order to corroborate our conclusions. Small beet plots were sown in Colonia Alvear, where the writer resided during the following summer (November–February). These plots were kept under close observation. Efforts were made to locate the breeding grounds of the insect in these regions. It was found at this time that the insect was present quite abundantly on the mud flats of the river Atuel, which passes through Colonia Alvear. Here the vegetation was of the salt marsh type and vegetation was more abundant than in the desert. However, a few specimens of *Eutettix tenella* were also found in the fields. The beets in the experimental plots came up and grew luxuriantly, as the soil is very fertile. No disease was observed until the beets were fully covering the ground. At this time a few beets began to have the watery translucent veins on the youngest leaves. Five days later, more than fifty per cent of the beets had developed the disease with all the characteristics which could be desired for identification. Ten days later only a few beets could be found which did not show some of the symptoms. So bad was the infection that a great number of the beets died.

By this time, *Eutettix tenella* was abundant both on the beet plots and in the adjacent bean fields. They were all full-grown specimens and rather light grey in color. On the desert vegetation, which was now drying up, many nymphs were to be found. The condition was about the same as the writer has observed many times around Visalia and also in the Salinas Valley (California).

The habitat and the habits of *Eutettix tenella* appear to be the same in South America as in North America. From Buenos Aires southward along the coast to Bahia Blanca, westward across the continent to Colonia Alvear and Mendoza, one meets climatic and regional conditions about the same as along the coast and in the valleys of California. *Eutettix tenella* is met with over those vast regions and this seems to be in its natural habitat. The habits of the insect and its relation to plant life seem to be the same in both continents. The insect moves from one aspect of vegetation to the other as they succeed during the season. It wants fresh growth for its nourishment and the plants which have the youngest green are usually the plants upon which it feeds. If in the middle of a desert a cultivated beet field appears at the time the

natural vegetation is drying up, the *Eutettix tenella* moves towards this young growth as occurred on our experiment plot in Guatrache.

Specimens of *Eutettix tenella* were caught and preserved in alcohol. They were identified by Mr. E. P. Van Duzee of the California Academy of Sciences, San Francisco, as being identical with our species, and specimens were deposited in the collection of the Academy. The insect in its local habitat seems to migrate from the foothills of the Andes and to invade the desert region following up the green vegetation after the winter rains have ceased. The insect has been found around Buenos Aires, in Guatrache, Alpachiri and Bahia Blanca (South-East), in Colonia Alvear and Mendoza (West), covering the major part of the temperate and sub-tropical zones of the Argentine Republic.

Specimens of diseased beets were also brought to the University of California for determination of the disease by Professor R. E. Smith of the Division of Plant Pathology. The insects and diseased plants were also examined and identified by Dr. H. H. Severin of the Division of Entomology.

PHYTOPATHOLOGICAL NOTES

Apple bitter-rot cankers in the eastern United States. In U. S. Department of Agriculture Bulletin 684, published in 1918, the writer stated that after careful search he had been unable to find bitter-rot cankers in the apple orchards of the eastern United States. He also noted that he had found no record of the occurrence of bitter-rot cankers in this region. On April 4, 1921, Dr. Errett Wallace of the Bureau of Plant Industry, U. S. Department of Agriculture, collected near Cornelia in northern Georgia, two diseased apple branches which he turned over to the writer. These branches bore typical bitter-rot cankers which, after remaining in moist chambers for 18 hours, were covered with the pinkish acervuli so characteristic of *Glomerella cingulata*. The spores, also, were in every way typical. On artificial media growth and development were the same as for authentic cultures from diseased fruits. Inoculation of healthy apples produced typical bitter-rot. In so far as the writer has knowledge of the subject, this is the first time that bitter-rot cankers have been reported from an Atlantic Coast State. The cankered branches have been deposited in the Office of Pathological Collections, Bureau of Plant Industry, U. S. Department of Agriculture. —JOHN W. ROBERTS.

Plum blotch. The attention of those interested in the plum disease caused by *Phyllosticta congesta* and described by the writer in the Journal of Agricultural Research, vol. 22, is called to the following paragraph on page 95 of the Cornell Agricultural Experiment Station Bulletin 38, "The cultivated native plums and cherries" by L. H. Bailey, published in 1892.

> "A fruit spot (Fig. 12) has been sent me by T. V. Munson, Denison, Texas, on the Golden Beauty. The disease is said to be serious in some years. It does not injure the fruit greatly except to disfigure it and to render it unmarketable. I submitted the specimens to Prof. J. E. Humphrey, of the Mass. Agricultural College, who found the disease to be a phoma, but the exact species could not be determined. It is, no doubt, somewhat allied to the black-rot of the grape, and the treatment used for grapes should be tried upon the plum."

The disease mentioned by Bailey is undoubtedly blotch. Fig. 12 is a line cut reproduction of a pen drawing showing a plum with numerous spots closely resembling blotch. As Phyllosticta and Phoma are closely

allied genera and as forms are commonly placed in one or the other according to their situation with regard to the host, Humphrey's identification of the fungus is easily understood. The very close resemblance between the Phoma stage of the grape black-rot pathogen and *Phyllosticta congesta* probably suggested the possibility of relationship between the two diseases. The Golden Beauty is a variety propagated from a plum found growing wild in western Texas and belongs to the Wayland group of *Prunus hortulana*. The finding of the disease on this variety as long ago as 1892 suggests the possibility that it may be indigenous and may have passed from wild plums to cultivated varieties and to other species such as *Prunus triflora* on which it was found in Georgia. This possibility is further supported by the fact that *Phyllosticta congesta* was originally described by Heald and Wolf as occurring on plum leaves in Texas.—JOHN W. ROBERTS.

Brown rot of apple. During the past winter the writer was making cultures from a considerable number of Stayman winesap apples for the purpose of securing cultures of *Glomerella rufomaculans* and was very much surprised to find that in nearly every case the decay was due either to Botrytis or Sclerotinia. Eight strains of the former and three of the latter were grown on four different media and carefully studied. In the case of Botrytis the strains showed very slight variations; in fact there was not as much variation between the different strains when grown on the same medium as between cultures of the same strains on different media. The conidiospores were mostly spherical and about 16 μ in diameter but some of them were elongated and measured 16 × 20 μ. Sclerotia were formed most abundantly on the oatmeal agar and ranged from one mm. in diameter to those that were 5 × 8 mm. in size. The Sclerotia cultures showed a great variation in vigor of growth and in size of conidiospores. The weakest grower developed spores ranging from 8 × 12 to 6 × 16 μ; the next stronger developed spores measuring from 20 × 20 to 24 × 36 μ; while the strongest grower developed spores measuring from 16 × 20 to 24 × 28 μ.—MEL. T. COOK.

A greenhouse disease of melons. During the winter of 1922–23, the attention of the writer was called to a disease of muskmelons in a greenhouse in northern New Jersey. The seeds were imported from England and the plants were grown on trellises, each plant being allowed to bear only one fruit. The disease was designated as "streak" by the gardener because that term very aptly described the symptoms. The gardener informed the writer that the first evidence of the disease would appear at any point on the vine and work in both directions as a dead brown

streak in the stem and frequently work out into the leaves. Pure cultures were obtained of a Fusarium very similar to *F. vasinfectum* but no inoculations were made to determine its pathogenicity. The question as to whether the organism was in the soil or in the seed could not be answered but it was probably the former.—MEL. T. COOK.

A serious disease of wheat caused by Sclerotium rhizodes in Idaho.—Early in the spring of 1922 specimens of a sclerotium disease on winter wheat was sent to the writer from Fremont County, Idaho. The disease was reported to be generally present in wheat fields aggregating about 300 acres and appeared to be causing considerable damage to wheat over that area. The original report of the disease stated that effected plants were covered with yellow or brown bodies and that when the snow melted these plants were covered with a mouldy growth. The disease appeared to be most severe where the snow had remained on the ground late in the spring. As the spring was exceedingly backward many farmers had attributed the injury to the heavy blanket of snow remaining so long on the ground.

Many of the plants which were sent in to the Experiment Station at Moscow were entirely dead. Others were only partially dead, the inner leaves still remaining a light green. The lower portions of these green leaves were often blackened and in some cases brown spots appeared upon these leaves. The bodies referred to above which according to reports were yellow or brown earlier in the season were found to be sclerotia and were a very dark brown or in most cases black when the specimens were received. They were scattered over the surfaces of the dead and dying leaves to which they were attached very securely. It was found practically impossible to separate them from the leaf surface without tearing the leaf. They were also present on the roots for one or two inches below the crown, and were found in great numbers embedded inside of the leaf sheath. They were about the size of the head of a small pin, spherical, or somewhat oval and flattened when dry and wrinkled or corrugated over their surfaces. They were very numerous on dead plants and on the dead leaves of plants which were partially killed. Twenty-five sclerotia were counted in one inch of the leaf of a dead plant.

As the writer was not familiar with the disease and did not have available facilities for determining it, specimens were sent to the Office of Pathological Collections of the United States Department of Agriculture for determination. The sclerotia were identified as sclerotia of *Sclerotium rhizodes* Awd. Miss Vera K. Charles of the Office of Patho-

logical Collections states in a letter regarding the matter that a similar specimen was sent in by Prof. R. A. Cooley of the Experiment Station at Bozeman, Montana in 1907.

The writer visited the region where the disease occurred late in June and made a rather careful survey of infected fields. The disease was found in a number of fields, the losses in the various fields ranging from a trace to fifty per cent in some cases. The disease appeared in spots in the fields and in these spots the majority of the plants were killed. The old dead plants could still be found on the ground where they had been killed in the rosette stage of growth. Around the margin of these spots as well as scattered thru the fields could be found plants which were only partially killed. These plants usually had but one or two weak stalks which often had failed to produce heads.

Thus far the present season the disease has not been reported. A careful survey wi'l be made for it this year and careful investigation will be undertaken should it appear again.—CHAS. W. HUNGERFORD.

Personals: Dr. John Asbury Elliott, Professor of Plant Pathology in the University of Arkansas and Plant Pathologist for the Arkansas Agricultural Experiment Station, died Thursday, January 18, at the Homeopathic Hospital, Washington, D. C. While at the Boston meetings of the American Phytopathological Society he became ill, and, after coming to Washington, pneumonia developed. The funeral services and burial were held January 20, at Rock Creek Cemetery in Washington, D. C.

Dr. Elliott held degrees from Fairmount College, the University of Kansas and the University of Illinois. From May 1916 to July 1917 he was Assistant Plant Pathologist at the Delaware Experiment Station, and from July 1917, until the time of his death he was the head of the Department of Plant Pathology at the University of Arkansas. He was thirty-five years old and leaves a wife and two children.

The September number of Phytopathology was issued September 18, 1923.